Available online at www.sciencedirect.com
SCIENCE DIRECT®
Analytica Chimica Acta 513 (2004) 141–150
ANALYTICA CHIMICA ACTA
www.elsevier.com/locate/aca

Comparative analysis of volatile compounds of 'fino' sherry wine by rotatory and continuous liquid–liquid extraction and solid-phase microextraction in conjunction with gas chromatography-mass spectrometry

R. Castro*, R. Natera, P. Benitez, C.G. Barroso

Analytical Chemistry Department, Faculty of Sciences, University of Cádiz, P.O. Box 40, Puerto Real, E-11510 Cádiz, Spain

Received 10 July 2003; received in revised form 26 November 2003; accepted 2 February 2004

Available online 12 March 2004

Abstract

Different headspace solid-phase microextraction (HS-SPME) methods have been selected and applied to the analysis of volatile compounds in 'fino' sherry wine by gas chromatography-mass spectrometry. A method based on rotary and continuous liquid–liquid extraction (LLE) for analysis of these same compounds has been optimised. The best conditions to extract this type of compounds using SPME and LLE were determined and both methods were validated. Both methodologies show adequate detection and quantitation limits, and linear ranges for correctly analysing these compounds. The recoveries obtained were close to 100%, with good repeatability values. The analytical and procedural advantages and disadvantages of these two methods have been compared. In general, SPME presented higher sensitivities. Both analytical methods were used to analyse five samples of 'fino' sherry wine supplied by different producers. No significant differences were found between the techniques at a significance level of 5%. The regression coefficients (r^2) for analysis using LLE and SPME exceeded 0.94 for all compounds. The LLE procedure is a method with high repeatability and has the possibility of simultaneous extraction of several samples (up to 12), however the SPME technique is a solvent-free method presenting major advantages, such as small sample volume and higher sensitivity and simplicity.

Keywords: Liquid–liquid extraction; Solid-phase microextraction; Volatile compounds; 'Fino' sherry wine

1. Introduction

Volatile compounds play an important role in the organoleptic characteristics of wines. Several hundred compounds from different families, such as alcohols, esters, aldehydes, ketones, volatile acids, terpenes, etc., contribute to wine flavour. This great variety of volatile compounds with different polarities, volatilities and wide range of concentration ensures that the flavour of a wine is very complex. The combination of all these compounds constitutes the character of wine and differentiates one wine from another [1]. In the case of 'fino' wines typical of the Jerez-Xérès-Sherry and Manzanilla de Sanlúcar Denomination of Origin (SW Spain), their chemical and organoleptic properties are established by the special system of aging in cask under the 'veil of flor' [2].

The analysis of volatile compounds is normally carried out by gas chromatography (GC) after previous extraction and concentration. Yet even today, the extraction and concentration of flavour components, prior to their analysis, constitute a problem that has still not been satisfactorily resolved. Classical analytical methods, such as liquid–liquid extraction (LLE) [3], static and dynamic headspace, simultaneous distillation-solvent extraction [4], and solid-phase extraction [5] have been widely used. Others methods, for example, involving ultrasound [6], supercritical fluid extraction [7], purge and cold trapping [8], and solid-phase microextraction (SPME) have also applied for analysing the volatile content of wines.

In recent years, different extraction methods based on solid-phase microextraction have been applied to analyse certain types of volatile compounds in wines [9–17]. Vas

* Corresponding author. Tel.: +34-56-01-63-63; fax: +34-56-01-64-60.
E-mail address: remedios.castro@uca.es (R. Castro).

0003-2670/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.aca.2004.02.002

et al. [13] reported the use of SPME for fast screening of different wine types. Whiton and Zoecklein [14] carried out the optimisation of headspace-SPME for the analysis of ten wine aroma compounds. Specific trace compounds, such as biacetyl [15], volatile and low volatile sulfides and disulfides [16], 2,4,6-tricloroanisole [17], etc., have been determined after SPME in wine.

Lately, a new technique, namely stir bar sorptive extraction (SBSE) [18], has also been developed. SBSE is more sensitive and can be used for trace and ultratrace analysis, while SPME is ideally appropriate for the analysis of compounds present at higher concentrations.

In spite of this great variety of analytical methods, liquid–liquid extraction continues to be the reference technique for the extraction of volatile compounds from wine [19–21]. In this sense, in previous studies carried out by our team [22], a rotary and continuous liquid–liquid extraction method with diethyl ether was optimised to analyse for polyphenolic compounds in wine. This technique permits the simultaneous extraction of 12 samples and minimises the appearance of analytical artefacts. Besides, in the case of polyphenolic compounds, good recoveries and R.S.D. values were obtained [22].

In this paper, the optimisation of rotary and continuous liquid–liquid extraction and the selection of a SPME method for analysis of volatile compounds in 'fino' sherry wine have been carried out. Then, both analytical methodologies have been validated and comparatively applied to the study of the volatile composition of 'fino' sherry wines. The analytical and procedural advantages and disadvantages of these two methods have been evaluated.

2. Experimental

2.1. Samples

2.1.1. Wine samples

A commercial 'fino' sherry wine was used to optimise the conditions of rotary and continuous liquid–liquid extraction and SPME for detection and quantification of volatile compounds.

'Fino' sherry wines from different wineries were also studied in order to compare both analytical methods previously mentioned.

2.1.2. Chemicals and reagents

All standards used in this study were supplied by Sigma-Aldrich (St. Louis, MO). 4-Methyl-2-pentanol was employed as internal standard. Ethanol, NaCl, anhydrous sodium sulfate, diethyl ether and *n*-pentane were purchased from Scharlau (Barcelona, Spain).

Individual stock standard solutions of each aroma compound were prepared by weight in ethanol. A global stock standard solution containing all the analytes was prepared in a synthetic wine matrix ($3\,g\,l^{-1}$ tartaric acid, and $15\,ml\,l^{-1}$ ethanol, in Milli-Q water). Working solutions used in further studies were prepared by diluting different amounts of the global standard solution in the synthetic wine solution. All these solutions were stored at 4 °C.

2.2. SPME

2.2.1. Parameter selection

Taking into account the data from the bibliography [9–12], five SPME methods were selected and applied to the particular problem of 'fino' sherry wine. Some modifications of these methods were also carried out in order to study the possible increase of the SPME efficiency. All these studies were carried out in triplicate.

The SPME conditions studied are shown in Table 1.

2.2.2. Selected SPME parameters

After selection, and for each SPME analysis, 25 ml of sample (natural or synthetic wine) was pipetted and placed into a 50 ml glass vial with 3.0 g of NaCl. Each sample was spiked with 75 μl of a solution of 4-methyl-2-pentanol ($2.516\,g\,l^{-1}$ in Milli-Q water containing 15% (v/v) of ethanol). A small magnetic stirring bar was also added. The vial was tightly capped with a PTFE-faced silicone septum and placed in a thermostatted block on a stirrer. The sample was equilibrated for 15 min at sampling temperature (40 °C) and, after this, the SPME fibre was inserted into the headspace. During the sampling time (45 min), the sample was stirred at constant speed. After completion of sampling, the fibre was removed from the sample vial and inserted into the GC injection port.

2.2.3. Matrix effect

For studying the possible effect of the ethanol content on headspace SPME, solutions from two extractions were analysed for each of the five synthetic wine samples ($3\,g\,l^{-1}$ tartaric acid in Milli-Q water with different ethanol contents (0, 5, 10, 15 and 20% (v/v)) and spiked with the same amounts of volatile compounds: alcohols (3-methyl-1-butanol,1-hexanol, 2-phenylethanol and 4-ethylguaiacol), esters (ethyl butanoate, isoamyl acetate, hexyl acetate, *cis*-3-hexenyl acetate, methyl octanoate, ethyl octanoate, diethyl succinate and phenylethyl acetate), and acids (butanoic and decanoic acid).

2.3. Rotary and continuous liquid–liquid extraction

2.3.1. Condition selection

The extraction device consists of an electric motor geared for low revolutions per min, to which a series of metal rods provided with clamps to hold the glass extraction ampoules are attached [22]. Twelve extractions can be carried out simultaneously into two glass ampoules, with an approximate volume of 150 ml, joined by a glass tube of 30 cm (body of the extractor). For each complete rotation, the extracting agent flows completely twice through the glass ampoules.

Table 1
Studied SPME conditions

Method	Type of fibre	Sample volume (ml)	Vial volume (ml)	Sampling temperature (°C)	Sampling time (min)		NaCl (g)
					Equilibrium time	Extraction time	
1	PDMS (100 μm)	25	50	25	15	15	6
2	CAR/PDMS (85 μm)	25	50	25	30	30	3
3	PA (85 μm)	25	50	25	15	15	6
4	PA (85 μm)	5	10	25	5	5	1
5	PDMS (100 μm)	12	20	30	–	30	–
6	PDMS (100 μm)	25	50	40	15	15	6
7	CAR/PDMS (85 μm)	25	50	40	30	30	3
8	CAR/PDMS (85 μm)	25	50	40	15	45	3
9	CAR/PDMS (85 μm)	25	50	25	15	45	3

The extraction procedure was optimised by applying a multilevel factorial design. In this, the experimental factors were extraction time (five levels), organic phase composition (three levels), and the ionic strength of the medium (two levels). Two experimental responses were studied, total chromatographic area and number of chromatographic peaks. This design involves 30 experiments undertaken in random order to provide protection against the effects of lurking variables. These experiments were carried out in duplicate. The values corresponding to the high (+) and low (−) points for each factor are shown in Table 2. For data manipulation, the Statgraphics Statistical Computer Package 'Statgraphics Plus 5.0' for Windows 98 was used.

For this process of optimisation, a commercial 'fino' sherry wine was employed. One hundred millilitres of sample diluted to 200 ml with Milli-Q water was used for each experiment. Diethyl ether and *n*-pentane in different proportions (1:1, 2:1 and 3:1) were employed as solvents. In all experiments, the total volume of extracting agent employed was 90 ml. This volume was selected taking into account the operational characteristics of the extraction device and the results obtained in a previous study (data not shown). Diethyl ether and *n*-pentane, together with dichloromethane, are the usual organic solvents employed to determine volatile compounds by liquid–liquid extraction [20,23].

2.3.2. Selected conditions

After optimisation, and for each analysis, 100 ml of sample (natural or synthetic wine) diluted to 200 ml with Milli-Q water and saturated with sodium chloride was placed into the body of the extractor. Each sample was spiked with 50 μl of a solution of 4-methyl-2-pentanol (10.035 g l^{-1} in Milli-Q water containing 15% (v/v) of ethanol). Ninety millilitres of diethyl ether:*n*-pentane (2:1, v/v) was added. The system was rotated at 0.8 rpm for 150 min. The organic phase, after drying for 15 min with anhydrous sodium sulfate, was concentrated in a Turbovap (Zymark, Hopkinton, MA) under a flow of nitrogen at room temperature to a final volume of 1 ml.

2.4. Chromatography

The samples were analysed using a GC 8000 chromatograph with a flame ionization (FID) detector (Fisons Instruments, Milan, Italy). The injection was made in the splitless mode for 2 min. The GC injection port temperature was 280 °C for all injections, except when PDMS fibres were used (250 °C in this case). The chromatograph was equipped with a DB-Wax capillary column (J & W Scientific, Folsom, CA), 60 m × 0.25 mm i.d., with a 0.25 μm coating. The carrier gas was helium at a flow rate of 1.1 ml min^{-1}. The detector temperature was 250 °C. The GC oven was programmed as follows: held at 35 °C for 10 min, then ramped at 5 °C min^{-1} to 100 °C. Then it was raised to 210 °C at 3 °C min^{-1} and held for 40 min.

The compounds were identified by mass spectrometric analysis. In these analyses, the same chromatograph coupled to a MD 800 mass detector (Fisons Instruments, Milan, Italy) was used. The mass detector operated in EI+ mode at 70 eV in a range of 30–450 amu. GC analytical conditions were the same as described above.

Table 2
LLE optimisation: factor levels

Factors	Factor levels			*F*-statistic and sign			
	Low	High	Number of levels	Total area		Number of peaks	
				F	Sign	*F*	Sign
Extraction time (min)	125	310	5	0.21	−	2.21	−
Ether:*n*-pentane (v/v)	1:1	3:1	3	0.02	+	0.05	+
NaCl	−	Saturation	2	4.45	−	0.14	+

The signal was recorded and processed with Masslab software supplied with the Wiley 6.0 MS library. Peak identification was carried out by analogy of mass spectra and confirmed by retention indices of standards. Quantitative data from the identified compounds were obtained by measuring the relative peak area in relation to that of 4-methyl-2-pentanol, the internal standard.

3. Results and discussion

3.1. SPME parameter selection

Fig. 1 shows the mean values obtained for number of chromatographic peaks and total chromatographic area after applying the SPME conditions detailed in Table 1 to a sample of 'fino' sherry wine. For both experimental responses, number of chromatographic peaks and total chromatographic area, the better results were obtained for method 8. A large number of chromatographic peaks were also obtained for method 7. As can be seen in Table 1, the sampling temperature for both methods was 40 °C, while a longer extraction time was used for method 8.

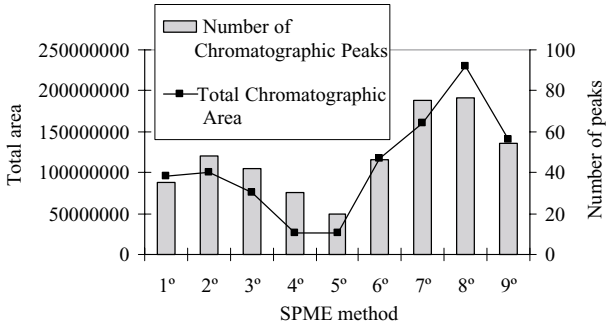

Fig. 1. Mean values obtained for the number of chromatographic peaks and total chromatographic area, after applying the SPME conditions detailed in Table 1 to a sample of 'fino' sherry wine.

Zhang et al. [24] pointed out that an increase in sampling temperature increases the headspace concentration of aroma compounds, favouring their extraction, but SPME involves an exothermic process and the extraction of analyte

Table 3
SPME: characteristics of the calibration graphs

Compound	Linear range (mg l^{-1})	Regression coefficient[a]	Linearity (LOL, %)	Slope ± S.D.	Intercept ± S.D.
Ethyl acetate	0.790–98.00	0.9982	98.65	0.092 ± 0.0012	−0.156 ± 0.0540
Ethyl butanoate	0.0601–2.76	0.9969	98.03	1.059 ± 0.0210	0.098 ± 0.027
Ethyl pentanoate	0.0099–0.993	0.9980	98.59	1.671 ± 0.0236	−0.010 ± 0.0102
Isoamyl acetate	0.0132–3.42	0.9988	98.91	1.429 ± 0.0155	0.045 ± 0.0089
2-Methyl-1-butanol	0.0901–150.02	0.9998	99.61	0.058 ± 0.0002	−0.001 ± 0.0022
Isoamyl alcohol	2.00–271.48	0.9987	98.74	0.060 ± 0.0008	0.060 ± 0.0366
Hexyl acetate	0.0171–1.72	0.9980	98.58	7.203 ± 0.1022	0.815 ± 0.0759
3-Hydroxy-2-butanone	1.61–201.6	0.9995	99.26	0.003 ± 0.0000	−0.001 ± 0.0021
cis-3-Hexenyl acetate	0.0159–1.59	0.9963	98.08	3.890 ± 0.0748	0.392 ± 0.0517
Ethyl lactate	0.1443–31.5	0.9963	97.85	0.006 ± 0.0001	0.001 ± 0.0021
1-Hexanol	0.0640–1.02	0.9988	98.76	0.353 ± 0.0044	0.059 ± 0.0063
Methyl octanoate	0.0155–1.54	0.9983	98.68	96.286 ± 1.2691	0.243 ± 0.8499
cis-3-Hexen-1-ol	0.0173–1.73	0.9978	98.52	1.222 ± 0.0181	0.185 ± 0.0136
Ethyl octanoate	0.0500–2.40	0.9955	97.31	80.004 ± 3.0309	10.397 ± 1.295
Acetic acid	0.348–92.8	0.9977	98.63	0.016 ± 0.0002	0.058 ± 0.0081
2-Furancarboxaldehyde	0.0501–2.40	0.9972	98.13	0.966 ± 0.0181	0.092 ± 0.0206
Benzaldehyde	0.0223–0.742	0.9984	98.61	9.434 ± 0.1316	0.237 ± 0.0467
2,3-Butanediol	0.1121–109.9	0.9968	98.37	0.035 ± 0.0006	0.152 ± 0.0253
Methyl decanoate	0.0156.1.56	0.9907	98.41	5.551 ± 0.1704	1.758 ± 0.1152
Ethyl 2-furoate	0.0186–1.85	0.9998	99.55	3.492 ± 0.0156	0.110 ± 0.0125
Butanoic acid	0.0457–1.53	0.9977	98.30	1.512 ± 0.0257	0.046 ± 0.0188
Isobutyric acid	0.5338–8.34	–	–	–	–
Ethyl decanoate	0.0501–2.25	0.9965	97.90	249.460 ± 5.25	7.660 ± 5.61
Diethyl succinate	0.0948–31.6	0.9947	97.97	0.133 ± 0.0034	0.390 ± 0.0520
Ethyl-2-phenyl acetate	0.0141–1.41	0.9976	98.57	4.670 ± 0.0667	0.155 ± 0.0430
Phenylethyl acetate	0.0191–1.91	0.9997	99.48	4.169 ± 0.0213	0.028 ± 0.0176
Hexanoic acid	0.1623–20.44	0.9997	99.41	0.334 ± 0.0020	−0.027 ± 0.0072
γ-Butyrolactone	0.0502–10.78	–	–	–	–
Benzyl alcohol	0.0178–1.78	0.9989	98.97	0.130 ± 0.0013	0.003 ± 0.0010
2-Phenylethanol	0.8002–39.5	0.9986	98.65	0.208 ± 0.0028	0.329 ± 0.0524
4-Ethylguaiacol	0.0128–1.28	0.9992	99.10	1.370 ± 0.0124	−0.015 ± 0.0069
Octanoic acid	0.1200–14.41	0.9985	98.64	1.157 ± 0.0158	−0.018 ± 0.0406
4-Ethylphenol	0.0168–1.68	0.9983	98.71	0.577 ± 0.0075	0.009 ± 0.0054
Decanoic acid	0.0507–6.24	0.9992	98.98	3.216 ± 0.0328	−0.088 ± 0.0365

[a] $n > 6$.

Table 4
LLE: characteristics of the calibration curves

Compound	Linear range ($mg\,l^{-1}$)	Regression coefficient[a]	Linearity (LOL, %)	Slope ± S.D.	Intercept ± S.D.
Ethyl acetate	0.790–98.00	–	–	–	–
Ethyl butanoate	0.0601–2.76	0.9978	97.66	0.0156 ± 0.0004	0.0016 ± 0.0005
Ethyl pentanoate	0.0800–1.30	0.9992	98.37	0.1384 ± 0.0023	−0.0025 ± 0.0017
Isoamyl acetate	0.0282–2.29	0.9973	98.35	0.0416 ± 0.0007	0.0031 ± 0.0008
2-Methyl-1-butanol	0.3843–130.02	0.9979	98.11	0.1050 ± 0.0020	−0.0066 ± 0.0095
Isoamyl alcohol	2.11–200.16	0.9971	98.08	0.1080 ± 0.0021	0.1922 ± 0.0974
Hexyl acetate	0.0366–1.72	0.9987	98.73	0.0777 ± 0.0010	0.0006 ± 0.0008
3-Hydroxy-2-butanone	2.24–182.12	0.9991	99.03	0.0036 ± 0.0000	−0.0055 ± 0.0030
cis-3-Hexenyl acetate	0.0340–2.76	0.9973	98.49	0.0662 ± 0.0010	0.0023 ± 0.0013
Ethyl lactate	2.01–94.43	0.9991	98.95	0.0253 ± 0.0003	−0.0248 ± 0.0120
1-Hexanol	0.0912–4.28	0.9986	98.66	0.1010 ± 0.0014	0.0060 ± 0.0028
Methyl octanoate	0.1837–8.61	0.9978	98.32	0.0506 ± 0.0008	−0.0034 ± 0.0035
cis-3-Hexen-1-ol	0.0339–1.59	0.9974	98.19	0.1793 ± 0.0032	0.0015 ± 0.0024
Ethyl octanoate	0.0310–2.52	0.9990	98.99	0.0619 ± 0.0006	−0.0002 ± 0.0008
Acetic acid	3.25–76.35	0.9988	98.57	0.0045 ± 0.0001	−0.0052 ± 0.0026
2-Furancarboxaldehyde	0.0501–4.92	–	–	–	–
Benzaldehyde	0.0475–2.23	0.9987	98.85	0.0978 ± 0.0011	0.0035 ± 0.0015
2,3-Butanediol	20.00–250.10	0.9993	98.37	0.0008 ± 0.0000	−0.0035 ± 0.0008
Methyl decanoate	0.0315.1.56	0.9983	98.37	0.0662 ± 0.0011	−0.0035 ± 0.0008
Ethyl 2-furoate	0.0790–1.85	0.9971	97.81	0.0935 ± 0.0020	−0.0025 ± 0.0020
Butanoic acid	0.5181–7.83	0.9984	98.37	0.0861 ± 0.0014	−0.0278 ± 0.0064
Isobutyric acid	0.5338–8.34	0.9966	97.76	0.0888 ± 0.0020	−0.0077 ± 0.0088
Ethyl decanoate	0.1280–2.25	0.9983	98.32	0.0518 ± 0.0009	−0.0054 ± 0.0009
Diethyl succinate	2.02–31.6	0.9983	98.56	0.0775 ± 0.0011	0.1026 ± 0.0502
Ethyl-2-phenyl acetate	0.0406–3.30	0.9971	98.29	0.1140 ± 0.0020	0.0086 ± 0.0031
Phenylethyl acetate	0.0600–1.41	0.9990	98.68	0.1411 ± 0.0019	−0.0035 ± 0.0014
Hexanoic acid	0.1656–7.76	–	–	–	–
γ-Butyrolactone	3.30–10.89	0.9988	98.61	0.0038 ± 0.0001	0.0060 ± 0.0022
Benzyl alcohol	0.0380–1.78	0.9968	97.99	0.1527 ± 0.0031	0.0194 ± 0.0026
2-Phenylethanol	0.8349–67.83	0.9962	98.04	0.1225 ± 0.0024	0.5817 ± 0.0779
4-Ethylguaiacol	0.0273–2.21	0.9970	98.26	0.1180 ± 0.0021	0.0021 ± 0.0022
Octanoic acid	0.1210–5.67	–	–	–	–
4-Ethylphenol	0.0640–5.20	0.9982	98.68	0.2336 ± 0.0031	−0.0068 ± 0.0077
Decanoic acid	0.0637–7.80	0.9980	98.43	0.0029 ± 0.0000	0.0007 ± 0.0002

[a] $n = 6$.

decreases as the temperature increases. In both methods, and taking into account that the type of fibre used has a high extraction capacity [12], a high temperature increased the experimental responses. This could be due to an increase of less volatile compounds in the gas phase that might compensate for the decrease of adsorption induced by this high temperature.

In summary, the best conditions to extract the aromatic compounds of 'fino' sherry wine were: type of fibre CAR/PDMS (85 μm), sampling temperature 40 °C, equilibrium time 15 min, extraction time 45 min, NaCl content 3.0 g, and sample volume 25 ml.

3.2. Rotary and continuous liquid–liquid extraction condition optimisation

The parameters optimised were extraction time, organic phase composition and ionic strength. The data obtained for these parameters were evaluated by analysis of variance (ANOVA) at the 5% significance level. Table 2 shows the F values and the sign of the effect of each factor on both chromatographic responses. The strength of the influence of a factor is indicated by the magnitude of the F value (variables with F values over 5.32 have a significant influence at the 5% significance level). None of these three parameters was shown to be significant (at $P < 0.05$) for both the number of chromatographic peaks and the total area in the region considered.

Organic phase composition (diethyl ether:*n*-pentane proportion) was the least influential variable that affected with a positive sign the values obtained for total area and number of peaks. The most influential factor for the total area, with a negative sign, was the ionic strength of the medium (addition until saturation of NaCl). In general, the modification of the nature of the matrix by adding a salt affects the liquid–liquid partition coefficients of the analytes, increasing their concentrations in the organic phase. However, if the analytes have a high polarity, the liquid–liquid extraction efficiency can be negatively affected.

For the number of chromatographic peaks, the most influential factor was extraction time. This factor showed a negative sign for both experimental responses.

Table 5
SPME: performance characteristics

Compound	Limit of detection (LOD, mg l^{-1})	Limit of quantitation (LOQ, mg l^{-1})	Recovery (%)	Repeatability (R.S.D., %)
Ethyl acetate	0.3090	1.0111	98.38	8.92
Ethyl butanoate	0.0234	0.0780	82.05	14.80
Ethyl pentanoate	0.0050	0.0120	84.00	9.72
Isoamyl acetate	0.0501	0.1660	100.22	3.83
2-Methyl-1-butanol	0.1510	0.5401	100.84	9.54
Isoamyl alcohol	0.2345	0.7567	86.97	12.52
Hexyl acetate	0.0050	0.0168	96.11	10.37
3-Hydroxy-2-butanone	2.0981	5.7811	106.28	13.44
cis-3-Hexenyl acetate	0.0273	0.0887	83.67	12.69
Ethyl lactate	0.0069	0.0245	91.69	8.70
1-Hexanol	0.0207	0.0671	80.52	4.80
Methyl octanoate	0.0104	0.0322	82.70	14.09
cis-3-Hexen-1-ol	0.0591	0.2901	81.41	13.46
Ethyl octanoate	0.0582	0.2870	84.66	13.56
Acetic acid	0.3004	1.0023	79.72	16.11
2-Furancarboxaldehyde	0.0359	0.1021	101.09	7.32
Benzaldehyde	0.0130	0.0381	88.17	12.81
2,3-Butanediol	0.1102	0.3012	80.85	13.25
Methyl decanoate	0.067	0.2021	81.65	7.84
Ethyl 2-furoate	0.0191	0.0592	82.10	13.63
Butanoic acid	0.0234	0.0712	115.83	9.89
Ethyl decanoate	0.0370	0.1098	87.84	14.49
Diethyl succinate	0.0091	0.0300	90.96	10.11
Ethyl-2-phenyl acetate	0.0125	0.0387	102.47	10.86
Phenylethyl acetate	0.0221	0.0723	91.86	14.89
Hexanoic acid	0.0661	0.0198	93.34	13.72
Benzyl alcohol	0.0125	0.0401	100.23	10.82
2-Phenylethanol	0.1456	0.4842	88.76	11.39
4-Ethylguaiacol	0.0103	0.0333	88.78	12.79
Octanoic acid	0.056	0.1842	113.72	12.42
4-Ethylphenol	0.0164	0.0523	100.12	8.46
Decanoic acid	0.0781	0.2601	105.96	11.39

As can be seen, for optimising both total chromatographic area and number of chromatographic peaks, a short of extraction time and a high proportion of diethyl ether:*n*-pentane are required. Moreover, in order to increase the number of volatile compounds extracted, a high ionic strength of the medium should be selected.

Summarising, based on the previous results and taking into account the values selected as optima by the statistical program used, the conditions for the continuous and rotary liquid–liquid extraction were chosen as follows: 100 ml of sample diluted to 200 ml with Milli-Q water; extraction time, 150 min; diethyl ether:*n*-pentane, 90 ml (2:1); and addition of NaCl until saturation.

3.3. SPME and rotary and continuous liquid–liquid extraction: performance characteristics

Five levels of concentration for each volatile compound, covering the concentration ranges expected, were tested in triplicate. The (volatile compound/internal standard) peak area ratio was used for each compound. The ranges of linearity studied appear in Table 3 for SPME and Table 4 for LLE. Excellent linearity was obtained for both methods and all volatile compounds ($r > 0.99$). It was also corroborated by the 'on-line linearity (LOL)' [25], with values >98% (Tables 3 and 4). This parameter is determined by the equation 'LOL (%) = 100 − R.S.D. (b)' in which R.S.D. (b) is the relative standard deviation of the slope (expressed as a percentage).

Some differences were found between both analytical methods. Hexanoic and octanoic acids, and 2-furancarboxaldehyde could not be found in the samples extracted by rotary and continuous liquid–liquid extraction while for ethyl acetate, the organic solvent peak impeded its determination. In the case of SPME, isobutyric acid and γ-butyrolactone could not be detected.

The slope of the straight calibration lines is a measure of method sensitivity and depends on both extraction efficiency and detector response for each compound. For LLE, the distribution of an analyte between two unmixed phases is an equilibrium phenomenon that depends on two groups of factors, one derived from the analytical process (volume ratio, ionic strength of the medium, etc.) and the other related to the characteristics of each compound, such as polarity, molecular structure and solubility in the aqueous matrix. In our case, low sensitivities were obtained for decanoic

Table 6
LLE: performance characteristics

Compound	Detection limit (LOD, mg l^{-1})	Quantitation limit (LOQ, mg l^{-1})	Recovery (%)	Repeatability (R.S.D., %)
Ethyl acetate	–	–	–	–
Ethyl butanoate	0.0312	0.0980	123.14	14.83
Ethyl pentanoate	0.0500	0.1667	124.89	4.06
Isoamyl acetate	0.0511	0.1700	114.23	4.42
2-Methyl-1-butanol	0.4411	1.4601	99.23	4.00
Isoamyl alcohol	0.3900	1.2871	105.23	5.69
Hexyl acetate	0.0144	0.0467	74.43	9.43
3-Hydroxy-2-butanone	2.02	6.05	108.13	7.82
cis-3-Hexenyl acetate	0.0341	0.1036	111.5	6.46
Ethyl lactate	0.0100	0.0313	114.91	5.15
1-Hexanol	0.0208	0.0678	116.23	4.98
Methyl octanoate	0.0841	0.2503	77.98	8.61
cis-3-Hexen-1-ol	0.0500	0.1523	82.12	5.70
Ethyl octanoate	0.0501	0.1434	105.42	3.79
Acetic acid	0.4511	1.3036	83.16	7.79
2-Furancarboxaldehyde	–	–	–	–
Benzaldehyde	0.0233	0.0707	97.84	7.96
2,3-Butanediol	0.9510	3.17	99.99	7.78
Methyl decanoate	0.0411	0.1371	84.95	12.01
Ethyl 2-furoate	0.0201	0.0570	99.49	5.70
Butanoic acid	0.0441	0.1450	118.35	5.30
Isobutyric acid	0.0911	0.3000	94.49	2.77
Ethyl decanoate	0.0544	0.1513	80.36	2.95
Diethyl succinate	0.0102	0.0351	103.04	1.65
Ethyl-2-phenyl acetate	0.0236	0.0756	109.32	5.20
Phenylethyl acetate	0.0341	0.1036	87.68	7.07
Hexanoic acid	–	–	–	–
γ-Butyrolactone	0.0544	0.1604	107.86	5.79
Benzyl alcohol	0.0236	0.0751	108.96	4.66
2-Phenylethanol	0.2221	0.7214	119.01	2.79
4-Ethylguaiacol	0.0154	0.0504	115.60	14.10
Octanoic acid	–	–	–	–
4-Ethylphenol	0.0196	0.0603	80.77	3.99
Decanoic acid	0.1564	0.5001	91.23	10.75

Table 7
Absolute peak areas obtained for synthetic wine samples with different ethanol content

Volatile compounds	Ethanol content (%)						
	0	5	10	15	20	Mean	R.S.D.
Isoamyl alcohol	2882852	2117553	2046461	2152843	1566745	1.01	4.31
1-Hexanol	1370714	874861	657645	463398	367734	3.25	0.39
2-Phenylethanol	7666766	5674402	5392856	2826698	2245192	2.47	2.50
4-Ethylguaiacol	1646485	1051297	882721	413958	196515	0.51	6.27
Ethyl butanoate	4888647	2168411	1398602	954917	616380	0.88	11.56
Isoamyl acetate	1343376	1015810	784205	707973	466564	0.48	8.85
Hexyl acetate	16931805	12002620	9102601	5841962	4671438	5.24	5.10
cis-3-Hexenyl acetate	12151055	7149353	5021986	3166004	2367233	2.96	7.45
Methyl octanoate	211706700	188124400	168333400	98899675	82329215	93.53	2.16
Ethyl octanoate	64896685	68988225	56164595	37666510	26411370	30.51	5.04
Diethyl succinate	15079795	10434820	8110418	5316403	4956956	4.79	3.51
Phenylethyl acetate	7666766	5674402	5392856	2826698	2245192	2.90	3.28
Butanoic acid	4353384	4106505	3340326	2790250	2394479	1.83	8.63
Decanoic acid	12711349	8913091	6101230	1977438	412141	2.74	5.60

Mean values of the relative areas (peak area/internal standard area) with their relative standard deviations (in %).

Table 8
Comparison of results of SPME and continuous and rotary liquid–liquid extraction (LLE) for the determination of volatile compounds in 'fino' sherry wine

Compound	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	y = SPME; x = LLE
Ethyl acetate	24.61	45.21	43.64	31.19	44.07	–
	–	–	–	–	–	
Ethyl butanoate	0.3041	0.4171	0.7612	0.8580	0.5861	$y = 1.1788x - 0.1051$
	0.3453	0.4655	0.7360	0.8172	0.5663	($r^2 = 0.9759$)
Ethyl pentanoate	nd	nd	0.2190	0.2375	nd	$y = 0.9850x + 0.0011$
	nd	nd	0.2161	0.2411	nd	($r^2 = 0.9905$)
Isoamyl acetate	1.48	0.1360	0.2621	0.1222	1.84	$y = 1.0066x + 0.0096$
	1.36	0.1284	0.2530	0.1421	1.88	($r^2 = 0.9887$)
2-Methyl-1-butanol	115.61	128.56	30.01	112.18	111.05	$y = 1.0065x + 0.0087$
	117.25	129.85	29.89	110.12	110.10	($r^2 = 0.9767$)
Isoamyl alcohol	63.16	279.27	235.56	74.19	130.30	$y = 0.9756x - 0.1980$
	65.18	283.56	232.14	73.14	128.45	($r^2 = 0.9812$)
Hexyl acetate	0.3500	0.2340	0.1201	0.0521	0.4144	$y = 1.0731x - 0.0097$
	0.3480	0.2226	0.1080	0.0748	0.3814	($r^2 = 0.9666$)
3-Hydroxy-2-butanone	4.25	4.97	16.09	10.09	7.54	$y = 1.0429x - 0.1447$
	4.22	4.73	14.79	10.79	7.30	($r^2 = 0.9470$)
cis-3-Hexenyl acetate	0.0926	0.0870	0.2498	0.1982	0.0711	$y = 1.0046x + 0.0012$
	0.0929	0.0793	0.2371	0.2074	0.0728	($r^2 = 0.9808$)
Ethyl lactate	26.02	31.76	105.97	66.09	26.22	$y = 1.0576x - 3.8345$
	31.25	32.09	104.00	64.88	28.02	($r^2 = 0.9858$)
1-Hexanol	1.94	2.51	1.98	0.7735	0.9695	$y = 0.9138x + 0.0207$
	2.03	2.81	2.09	0.9123	0.9954	($r^2 = 0.9598$)
Methyl octanoate	0.0918	0.1480	0.2103	0.1449	0.0965	$y = 0.8777x + 0.0164$
	0.0928	0.1307	0.2241	0.1543	0.0927	($r^2 = 0.9463$)
cis-3-Hexen-1-ol	0.2231	0.2161	0.1086	0.1980	0.4306	$y = 0.7705x + 0.0402$
	0.2303	0.2243	0.0977	0.2059	0.5075	($r^2 = 0.9848$)
Ethyl octanoate	0.5349	1.09	0.0279	0.0289	0.8447	$y = 0.9650x + 0.0078$
	0.5031	1.08	0.0298	0.0312	0.9337	($r^2 = 0.9837$)
Acetic acid	6.21	10.01	11.63	8.81	7.65	$y = 0.9154x + 0.7946$
	6.14	10.17	11.82	8.36	7.61	($r^2 = 0.9621$)
2-Furancarboxaldehyde	0.0413	0.8072	0.1847	0.0850	0.2107	–
	–	–	–	–	–	
Benzaldehyde	2.24	2.01	13.55	2.11	4.19	$y = 1.0445x - 0.4405$
	2.37	2.24	13.24	2.33	5.00	($r^2 = 0.9929$)
2,3-Butanediol	164.39	139.75	422.07	224.29	187.29	$y = 0.9475x + 21.292$
	128.85	125.09	409.26	225.13	200.19	($r^2 = 0.9586$)
Methyl decanoate	0.3209	0.1299	0.6465	0.4145	0.2931	$y = 1.0496x + 0.0009$
	0.2763	0.1446	0.6231	0.3707	0.3007	($r^2 = 0.9633$)
Ethyl 2-furoate	0.0838	0.0119	0.5349	0.4558	0.1290	$y = 0.9304x + 0.0027$
	0.0957	0.014	0.5242	0.5332	0.1248	($r^2 = 0.9722$)
Butanoic acid	0.9540	1.47	2.25	1.68	0.9437	$y = 0.9978x - 0.0402$
	1.04	1.58	2.31	1.66	0.9412	($r^2 = 0.9785$)
Isobutyric acid	–	–	–	–	–	–
	0.5755	0.4970	5.74	5.18	0.6562	
Ethyl decanoate	0.1863	0.2180	0.4266	0.3176	0.1976	$y = 1.3198x - 0.0815$
	0.2083	0.2238	0.3818	0.3065	0.2083	($r^2 = 0.9853$)
Diethyl succinate	0.2601	2.83	24.63	19.87	1.83	$y = 0.9122x + 0.6662$
	0.2716	2.28	28.39	17.57	2.00	($r^2 = 0.9705$)
Ethyl-2-phenyl acetate	0.0933	0.0194	0.1421	0.0932	0.1048	$y = 0.9672x + 0.0067$
	0.0833	0.0174	0.1452	0.0893	0.0983	($r^2 = 0.9852$)
Phenylethyl acetate	nd	nd	0.0414	0.0096	nd	$y = 0.9027x + 0.0000$
	nd	nd	0.0458	0.0102	nd	($r^2 = 0.9987$)
Hexanoic acid	2.53	7.57	1.16	1.09	2.66	–
	–	–	–	–	–	
γ-Butyrolactone	–	–	–	–	–	–
	7.30	5.72	10.58	17.84	7.20	
Benzyl alcohol	0.4409	0.6206	2.03	1.13	0.4388	$y = 0.9386x + 0.0342$
	0.4674	0.5300	2.11	1.16	0.5040	($r^2 = 0.9827$)
2-Phenylethanol	9.67	10.21	46.45	34.42	13.27	$y = 0.8525x + 2.0169$
	9.58	9.55	53.08	36.27	13.53	($r^2 = 0.9867$)
4-Ethylguaiacol	0.6362	0.0611	0.0376	0.1152	0.0395	$y = 1.0416x - 0.0077$
	0.6084	0.0577	0.0437	0.1354	0.0457	($r^2 = 0.9802$)

Table 8 (Continued)

Compound	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	y = SPME; x = LLE
Octanoic acid	5.03	10.07	0.4790	0.4488	5.89	–
	–	–	–	–	–	
4-Ethylphenol	0.9381	0.2489	0.0214	0.2803	0.2526	$y = 1.0351x$–0.0377
	0.9226	0.2639	0.0239	0.3534	0.3006	($r^2 = 0.9816$)
Decanoic acid	1.76	2.87	1.04	0.5288	0.3398	$y = 0.9995x - 0.0347$
	1.79	2.90	1.09	0.5214	0.4143	($r^2 = 0.9748$)

Mean values (mg l^{-1}). Upper value for each compound was obtained by SPME. nd: not detected.

acid, 3-hydroxy-2-butanone, acetic acid, 2,3-butanediol and γ-butyrolactone by this particular liquid–liquid extraction technique (Table 4). 3-Hydroxy-2-butanone, 2,3-butanediol and acetic acid also exhibited low sensitivities when they were extracted by SPME. In general, higher slopes were obtained for SPME.

Detection and quantitation limits (Tables 5 and 6) were calculated from the calibration plots (three and ten times, respectively, the relative standard deviation of the analytical blank values) constructed for each volatile compound and analytical method. The values obtained are, in general, low enough to permit the determination of these compounds in real wine samples (Tables 5 and 6), although, as can be expected, those obtained by LLE were higher than those obtained by SPME. Different authors have found a high sensitivity using SPME for analysing different analytes in several matrices [9,26,27].

The repeatability has been evaluated by means of three sets of five extractions of a global standard solution. The measurements ([analyte/internal standard] peak area ratio, $n = 5$) were found to be repeatable with R.S.D. values of 4–14% for SPME (Table 5) and 2–14% for LLE (Table 6).

In comparison with other isolation analytical techniques, López and Gómez [23], using SPE, found reproducibility values <10% for most of the volatile compounds considered. Ortega-Heras et al. [28] studied three extraction methods (liquid–liquid extraction, static headspace and a new method similar to the dynamic headspace technique) for the analysis for volatile components in wine. Liquid–liquid extraction and static headspace were more repeatable with R.S.D. values of 5–10%. De la Calle et al. [29] obtained R.S.D. values in the range 8–22% for analysis for terpenoids in wine using headspace-SPME. Ferreira et al. [30] developed a method based on SPE for the gas chromatographic analysis of wine volatiles with R.S.D. values ≤10%.

As can be seen, in our case, both methods generate repeatable results, similar to other analytical techniques.

The method of standard additions was used in order to check the accuracy of both analytical methods. A representative sample of 'fino' sherry wine was taken as the matrix and known quantities of a global standard solution containing all the analytes were added at five levels and in triplicate. The slopes of the lines thus obtained for each of the volatile compounds and method were compared with the corresponding slopes obtained in the calibration with standards (t criterion). No significant differences were found between them at a significance level of 5%. Tables 5 and 6 give the data for the recovery of each compound and method, determined by the slope of the line plotting the concentration found against the concentration expected. Recoveries near 100% were obtained for all the volatile compounds.

3.3.1. SPME: matrix effect

In wines, the ethanol content appears to interfere in the SPME technique but, for quantitative analysis, the compound area/internal standard area ratios may be used [14,28]. To check this source of possible interference, the same amounts of aroma compounds (alcohols, esters and acids) were added to five synthetic wine samples with different ethanol content. Three extractions were analysed for each of these synthetic samples. The data obtained show that the higher the ethanol concentration, the lower the extraction efficiency (Table 7). Although the absolute areas decrease, the compound area/internal standard area ratio remains constant and the relative standard deviations are <10%. In general, for quantitative analysis by SPME, the internal standard may be used, so the ethanol concentration does not affect the analytical data.

3.4. Determination of volatile compounds in wines: comparison of the two analytical methods

Both analytical methods were used to analyse five 'fino' sherry wines supplied by different producers. The results obtained for these samples are shown in Table 8. As can be seen, the values obtained for these compounds by SPME were similar to those obtained from rotary and continuous liquid–liquid extraction. The regression coefficients (r^2) for analysis by SPME and rotary and continuous liquid–liquid extraction always exceeded 0.95 (Table 8), indicating that results from both methods are in agreement.

In general, the concentrations obtained lie within the calibrated intervals. The major volatile compounds quantified were ethyl acetate, 2-methyl-1-butanol, isoamyl alcohol, 2,3-butanediol, ethyl lactate, 3-hydroxy-2-butanone, acetic acid, 2-phenylethanol, γ-butyrolactone, hexanoic acid and octanoic acid. Among these acids, butanoic, hexanoic and octanoic were found in the highest concentrations. Isobutyric acid was the acid found in the lowest concentration whereas hexanoic acid had the highest concentration.

Among the esters identified, the major compounds were ethyl acetate, ethyl lactate, diethyl succinate, and isoamyl acetate. Most of these compounds had concentrations in accord with those reported for other, similar wines [6,31].

Further researches, in which a higher number of 'fino' sherry wine samples will be studied, are required in order to obtain more information about the volatile profile of these white wines during and after their particular process of ageing in wood.

4. Conclusions

Both the methods used for determination of volatile compounds were adequate and sensitive, and the results were demonstrated to be in good agreement. The SPME showed higher sensitivities than LLE for several compounds. The LLE procedure provides the possibility of simultaneous extraction of several samples (up to 12); however SPME is a solvent-free method presenting major advantages, such as small sample volume and higher sensitivity and simplicity.

References

[1] C. García-Jares, S. García-Martín, R. Cela-Torrijos, J. Agric. Food Chem. 43 (1995) 764.
[2] J. Casas, Descripción resumida de la técnica enológica de los vinos de Jerez, in: Jimenez-Mena (Ed.), Proceedings of the University of Cadiz, III Jornadas Universitarias sobre el Jerez, vol. 1, University of Cadiz Press, Cadiz, 1985, pp. 333–361.
[3] A. Rapp, H. Hastrich, L. Engel, Vitis 15 (1976) 29.
[4] G.P. Blanch, G. Reglero, M. Herraiz, J. Tabera, J. Chromatogr. Sci. 29 (1991) 11.
[5] C.G. Edwards, R.B. Beelman, J. Agric. Food Chem. 38 (1990) 216.
[6] D. Hernanz, F.J. Heredia, R. Beltrán, M.A. Fernandez-Recamales, Talanta 50 (1999) 413.
[7] G.P. Blanch, G. Reglero, M. Herraiz, J. Agric. Food Chem. 43 (1995) 1251.
[8] M.R. Salinas, G.L. Alonso, F.J. Esteban-Infantes, J. Agric. Food Chem. 42 (1994) 1238.
[9] M. Mestres, C. Sala, M.P. Martí, O. Busto, J. Guasch, J. Chromatogr. A 835 (1999) 137.
[10] S. Francioli, M. Guerra, E. López-Tamares, J.M. Guadayoi, J. Caixach, Am. J. Enol. Vitic. 50 (1999) 404.
[11] A. Mallouchos, M. Komaitis, A. Koutinas, M. Kanellaki, J. Agric. Food Chem. 50 (2002) 3840.
[12] M. Mestres, M.P. Martí, M. Miracle, C. Sala, O. Busto, J. Guasch, Técnicas de Laboratorio 251 (2000) 289.
[13] G.Y. Vas, K. Köteleky, M. Farkas, A. Dobo, K. Vekey, Am. J. Enol. Vitic. 49 (1998) 100.
[14] R.S. Whiton, B.W. Zoecklein, Am. J. Enol. Vitic. 51 (2000) 379.
[15] Y. Hayasaka, E.J. Bartowsky, J. Agric. Food Chem. 47 (1999) 612.
[16] M. Mestres, M.P. Martí, O. Busto, J. Guasch, J. Chromatogr. A 881 (2000) 583.
[17] T.J. Evans, C.E. Butzke, S.E. Ebeler, J. Chromatogr. A. 786 (1997) 293.
[18] E. Baltussen, P. Sandra, F. David, C. Cramers, J. Microcol. Sep. 11 (1999) 737.
[19] J. Villen, F.J.D. Señoráns, G. Reglero, M. Herraíz, J. Agric. Food Chem. 43 (1995) 717.
[20] R. Schneider, R. Baumes, C. Bayonove, A. Razungles, J. Agric. Food Chem. 46 (1998) 3230.
[21] V. Lavigne, R. Henry, D. Dubourdieu, Sci. del Aliments 18 (1998) 175.
[22] E.R. Brú, C.G. Barroso, R. Cela, J.A. Pérez-Bustamante, Analyst 121 (1996) 297.
[23] E.F. López, E.F. Gómez, Chromatographia 52 (2000) 798.
[24] Z. Zhang, M.J. Yang, J. Pawliszyn, Anal. Chem. 66 (1994) 844.
[25] L. Cuadros, A.M. García-Campaña, J.M. Bosque, Anal. Lett. 29 (1996) 1231.
[26] J. Pino, M.P. Martí, M. Mestres, J. Pérez, O. Busto, J. Guasch, J. Chromatogr. A. 954 (2002) 51.
[27] M.N. Sarrión, F.J. Santos, M.T. Galceran, J. Chromatogr. A. 947 (2002) 155.
[28] M. Ortega-Heras, M.L. Gónzalez-SanJosé, S. Beltrán, Anal. Chim. Acta 458 (2002) 85.
[29] D. De la Calle, M. Reichenbächer, K. Danzer, J. High Resol. Chromatogr. 21 (1998) 373.
[30] V. Ferreira, M. Sharman, J.F. Cacho, J. Dennis, J. Chromatogr. A 731 (1996) 247.
[31] C. Ortega, R. López, J. Cacho, V. Ferreira, J. Chromatogr. A 923 (2001) 205.