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Analytical performance of three commonly used extraction methods for the gas chromatography–mass spectrometry analysis of wine volatile compounds

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ABSTRACT

The analytical performance of three extraction procedures based on cold liquid–liquid extraction using dicloromethane (LLE), solid phase extraction (SPE) using a styrene–divinylbenzene copolymer and headspace solid phase microextraction (SPME) using a carboxen–polydimethylsiloxane coated fibre has been evaluated based on the analysis of 30 representative wine volatile compounds. From the comparison of the three procedures, LLE and SPE showed very good linearity covering a wide range of concentrations of wine volatile compounds, low detection limits, high recovery for most of the volatile compounds under study and higher sensitivity compared to the headspace-SPME procedure. The latter showed in general, poor recovery for polar volatile compounds. Despite some drawbacks associated with the LLE and SPE procedures such as the more tedious sampling treatment and the use of organic solvents, the analytical performance of both procedures showed that they are more adequate for the analysis of wine volatiles.

1. Introduction

The volatile fraction of wine determines to a great extent its aroma, which is one of the most important characteristics influencing wine quality and consumer preferences. However, the wine volatile fraction is extremely complex, mainly because of the great number of compounds which form it. To date, more than 1000 compounds have been identified [1], which are from different chemical classes, covering a wide range of polarities, solubility and volatilities. In addition, the concentration range of these compounds in wines can be from a few ng L^{-1} to hundreds of mg L^{-1}. Moreover, volatile compounds are contained in complex and compositionally very variable matrices where they can be associated and therefore their volatility modulated by other wine macro-components (polyphenols, ethanol, polysaccharides) [2]. Finally, but also of importance, is the fact that many aroma compounds are chemically very unstable and can be easily oxidized or thermo degraded [3].

Therefore, the search of adequate extraction techniques allowing the identification and quantification of wine volatile compounds has attracted the attention of many scientists. This has resulted in the availability of a wide range of analytical tools for the extraction of these compounds from wine. These methodologies are mainly based on the solubility of the compounds in organic solvents (liquid–liquid extraction: LLE, simultaneous distillation liquid extraction: SDE), on their volatility (static and dynamic headspace techniques), or based on their sorptive/adsorptive capacity on polymeric phases (solid phase extraction: SPE, solid phase microextraction: SPME, stir bar sorptive extraction: SBSE). In addition, volatile compounds can be extracted by methods based on combinations of some of these properties (headspace solid phase microextraction HS-SPME, solid phase dynamic extraction: SPDE).

Some of the most commonly used methods for the analysis of volatile compounds in wine are LLE, SPE and SPME. Although LLE is being replaced by more manageable and solvent-free techniques, this type of extraction is still a reference for the analysis of wine aroma compounds [4–10]. The main advantages of this technique are its capacity to extract a wide range of compounds of different volatilities (as long as they have an affinity to the solvent), the high repeatability and the possibility of carrying out simultaneous extractions [11]. The possibility of using different sorbent phases and eluents makes SPE a very selective technique, and the fact that only minor amounts of organic solvents are used compared to LLE, is why SPE has been extensively used for the analysis of volatile aroma compounds [12–16] and off-flavours [17,18] in wines. However, the above-mentioned methods have started to be displaced by the SPME technique. Since its first application for the analysis of wine volatile compounds in the late 90s [19], its use for wine aroma analysis has been increasing. The high selectivity of the technique due to the commercial availability of many polymeric phases, its speed, simplicity, the fact that it is solvent-free, and the possibility of automatisation of the whole extraction process, are the main reasons for the great success of this technique for the analysis of different types of wine volatile compounds [20–28].

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Although many procedures for the analysis of wine volatile compounds have been published, as far we know, the comparison between the three extraction methodologies based on their analytical performance has not yet been carried out. Therefore, the objective of the present work was to compare the analytical performance of three of the most commonly used extraction procedures based on the LLE using dichloromethane as the organic solvent, SPE using a styrene–divinylbenzene copolymer and a SPME technique using a carboxen–polydimethylsiloxane fibre, for the gas chromatography mass spectrometry analysis of 30 representative wine volatile compounds.

2. Experimental

2.1. Synthetic wines

Synthetic wines were prepared by mixing 120 mL L^{-1} ethanol (VWR, Leuven, Belgium) and 4 g L^{-1} of tartaric acid (Panreac, Barcelona, Spain). The pH was adjusted to 3.5 with NaOH (Panreac).

A solution of methyl-nonanoate (1731-84-6) from Sigma–Aldrich (St, Louis, MO) was prepared in absolute ethanol HPLC grade (500 μL L^{-1}) and added to the synthetic wine to have a final concentration of 0.2 μL L^{-1}. This was used as the internal standard in the three extraction methods.

2.2. Wine samples

A commercial monovarietal red wine (*var*. Tempranillo), pH 3.6, was used for the recovery experiments.

2.3. Chemicals and reagents

An aroma standard solution formed with butyl acetate (123-86-4), ethyl hexanoate (123-66-0), ethyl decanoate (110-38-3) isovaleric acid (503-74-2) and vanillin (121-33-5) from Merck (Darmstadt, Germany); isobutyl acetate (110-19-0), ethyl butanoate (105-54-4), isopentyl acetate (123-92-2), hexyl acetate (142-92-7), acetoin (513-86-0), 1-hexanol (111-27-3), *cis*-3-hexen-1-ol (928-96-1), ethyl octanoate (106-32-1), furfural (98-01-1), linalool (78-70-6), γ-butyrolactone (96-48-0), diethyl succinate (123-25-1), α-terpineol (98-55-5), β-damascenone (23726-91-2), 2-phenylethyl acetate (103-45-7), geraniol (106-24-1), guaiacol (90-05-01), whiskey lactone (39212-23-2), β-ionone (79-77-6) and eugenol (97-53-0) from Sigma–Aldrich; hexanoic acid (142-62-1), octanoic acid (124-07-2) and decanoic acid (334-48-5) from Scharlau (Barcelona, Spain) and 4-ethyl guaiacol (2785-89-9) from Lancaster (Eastgate, White Lund, Morecambe, England), was prepared in HPLC grade absolute ethanol supplied by Merck. These compounds were selected for their important role for wine aroma [29–31]. All the aroma standards had a purity greater than 98%. The final concentration of each aroma compound in the standard solution was 500 mg L^{-1}. Working solutions used in order to determine the performance characteristics of the three extraction methods were prepared by diluting different amounts of the standard solution in a synthetic wine. All the solutions were stored at 4 °C.

2.4. Headspace solid phase microextraction (HS-SPME)

Eight milliliters of synthetic wine containing the aroma compounds were placed in a 20 mL headspace vial and sealed with a PTFE/Silicone septum (Supelco, Bellefonte, PA). Samples were left in a water bath at 40 °C for 10 min before the extraction. The extraction was performed with the exposure of a StableFlex 85 μm carboxen–polydimethylsiloxane, CAR–PDMS fibre (Supelco) to the headspace of the sample for 10 and 20 min at 40 °C and under constant stirring (500 rpm) or without stirring, depending on the experiment. After the extraction, the fibre was removed from the sample vial and desorbed in the GC injector port in splitless mode for 10 min. Six levels of concentration of each aroma compound (2, 10, 100, 500, 1000, 5000 μg L^{-1}), covering the concentration ranges expected in wines were tested in duplicate. Prior to use, the fibre was conditioned following the supplier's recommendation. The relative TIC responses of the 30 volatile compounds in the synthetic wines were compared to assess the effect of the extraction variables (stirring, extraction time).

2.5. Liquid–liquid extraction (LLE)

Fifty milliliters of synthetic wines containing the internal standard (methyl-nonanoate 0.2 μL L^{-1}) and the solution of aroma compounds were placed in a glass capped Erlenmeyer flask with a magnetic stirrer. The mixture was extracted with 10 mL dichloromethane (Merck) under continuous stirring in an ice bath for 1 h. Afterwards, the mixture was left in an ultrasound bath for 15 min to avoid the possible formation of an emulsion (in the real wine samples). After the liquid–liquid separation, the organic phase was collected and filtered through glass wool and dried over anhydrous sodium sulphate into a graduated flask. The extract was concentrated to 1 mL using a Vigreaux column in a 60 °C water bath and then, to a final volume of 300 μL under a helium stream. The extract was then hermetically capped and stored in a freezer (−25 °C) until GC–MS analysis. One microliter of extract was injected in the GC–MS in split mode (1:20). Seven levels of concentrations of each aroma compound (2, 10, 100, 500, 1000, 5000, 10,000 μg L^{-1}) were tested in duplicate.

2.6. Solid phase extraction (SPE)

Fifty milliliters of synthetic wine containing the internal standard (methyl-nonanoate 0.2 μL L^{-1}) and the solution of aroma compounds were passed through SPE cartridges (200 mg LiChrolut-EN resins) supplied by Merck at 2 mL min^{-1} using an extraction unit (VacMaster®, Biotage, Uppsala, Swedden). The cartridges were previously conditioned following the protocol described by Lopez et al. [15]. Analyses were carried out by elution with 1.3 mL dichloromethane (Merck). The extract was then hermetically capped and stored in a freezer (−25 °C) until the GC–MS analysis. One microliter of extract was injected in the GC–MS in split mode (1:20). The same seven levels of concentration of each aroma compound as for LLE were tested in duplicate.

2.7. Gas chromatography–mass spectrometry analysis

An Agilent 6890N GC system (Agilent, Palo Alto, CA) with a split/splitless injector and interfaced with an Agilent 5973N mass spectrometer was used for sample analysis. The injector was set at 250 °C (in the case of SPME analysis it was set at 280 °C). Agilent MSD ChemStation Software (D.01.02 16 version) was used to control the system. For separation, a Carbowax 10 M fused silica capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness) Quadrex Co. (Woodbridge, CT) was used. Helium was the carrier gas (7 psi). The oven temperature was programmed as follows: 40 °C as initial temperature, held for 5 min, the temperature, then increased to 200 °C at 4 °C/min, then held for 15 min.

For the MS system, the temperatures of the transfer line, quadrupole and ionization source were 270, 150 and 230 °C respectively; electron impact mass spectra were recorded at 70 eV ionization voltages and the ionization current was 10 μA. The acquisitions were performed in Scan mode (from 35 to 450 amu). Peak identification was carried out by analogy of mass spectra with those of the mass library (Wiley 6.0) and comparing the calculated retention indices with those published in the literature. Quantita-

tive data were obtained by calculating the relative peak area (or TIC signal) in relation to that of the internal standard (methyl-nonanoate).

2.8. Statistical analysis

The statistical methods used for the data analysis were: *t*-test to compare the relative TIC areas in the two SPME extraction procedures, linear regression to establish the calibration curves of each aroma compound with the three extraction procedures, and the lack of fit test to judge the adequacy of the models. STATGRAPHICS Centurion XV program, version 15.2 (2006, Statistical Graphics Corporation, Manugistics Inc., MD, www.statgraphics.com) was used for data processing.

3. Results and discussion

3.1. CAR–PDMS-SPME extraction conditions

SPME has become one of the most commonly used techniques for the extraction of volatile compounds from wines. However, most of the studies have been carried out using PDMS fibres [22,24,32–35]. Nonetheless, in recent years the use of different phases based on the combination of various adsorbent/absorbent polymers, such as DVB–CAR–PDMS or CAR–PDMS has been gaining popularity, since they can be used for the extraction of a broader range of analytes. Although they have not often been used to sample volatiles from wine, they are currently being used for the analysis of volatiles in different beverages and fermented foods, such as vinegar [36] whiskey [37] and Sherry wine [11]. In addition, the possibility of using StableFlex fibres coated on a flexible fused silica core, results in a more stable coating and a less breakable fibre. But the extraction selectivity may also be slightly different from the same coating on a standard fused silica core. Based on these antecedents, the performance of the StableFlex CAR–PDMS fibre for the analysis of volatile compounds in wine needs further investigation.

Therefore, in a first experiment, the influence of two extraction variables (stirring conditions and extraction time) was evaluated for the 30 volatile compounds contained in synthetic wines. It is generally accepted, that stirring usually improves the extraction of volatile compounds when using SPME [19,23,28], as the static layer resistant to mass transfer is usually destroyed, facilitating the mass transport between the bulk of the aqueous sample and the fibre [37]. By using *t*-test, the relative TIC area (TIC area compound/TIC area internal standard) of the 30 volatile compounds in the synthetic wines was studied to determine the effects of stirring at 250 rpm and without on the extraction procedure. Surprisingly, the results showed that stirring the sample did not produce a significant ($p > 0.05$) effect for most of the studied volatile compounds. In fact, only four compounds showed a significant increase ($p < 0.05$) in the relative TIC areas compared to the non-stirring conditions. These compounds were ethyl decanoate, γ-butyrolactone, diethyl succinate and octanoic acid. The two first compounds showed the highest increase in relative area (36% compared to the non-stirring conditions). Octanoic acid also showed an increase in the TIC area of above 30%, while diethyl succinate showed a slight but significant increase of 7.22% compared to the non-stirring conditions (data not shown).

Although stirring the sample only improved the extraction of some of the volatile compounds, we decided to keep these conditions, since the extraction of a greater amount of aroma compounds can be decisive in improving sensitivity when analysing real wine samples. Regarding the effect of the extraction time, the rationale for using these relatively low exposure times, was the possible saturation of the CAR–PDMS fibre with non-polar compounds like esters, which are quite abundant in real wines [20] as well as in the volatile blend under study. Results showed that this factor affected many of the studied aroma compounds, with 14 of the 30 volatile compounds being significantly influenced ($p < 0.05$). Fig. 1 shows these volatile compounds. For each compound, a relative area of 100% was assigned to the extraction conditions that gave the highest relative TIC area, while the areas obtained with the other extraction conditions were expressed as a percentage of the former. As it can be seen in the figure, for the 14 volatile compounds, the extraction yield was higher when employing 20 min of extraction. It is interesting to notice that most of these compounds corresponded to those exhibiting a higher retention index; therefore, most of them were compounds with relatively low vapour pressure values. For some of these compounds, such as ethyl decanoate, γ-butyrolactone, β-ionone, octanoic acid, eugenol and decanoic acid, the values of relative peak area when extracted for 20 min, were almost twice than when extracting for 10 min. However, Canuti et al. [21], have recently shown that neither the extraction temperature (40 or 50 °C) nor extraction time (30 or 60 min) influences the extraction yield of wine volatile compounds when using a PDMS fibre to analyse wine volatile compounds. The fact that the above-mentioned authors used total ion chromatogram signals (sum of

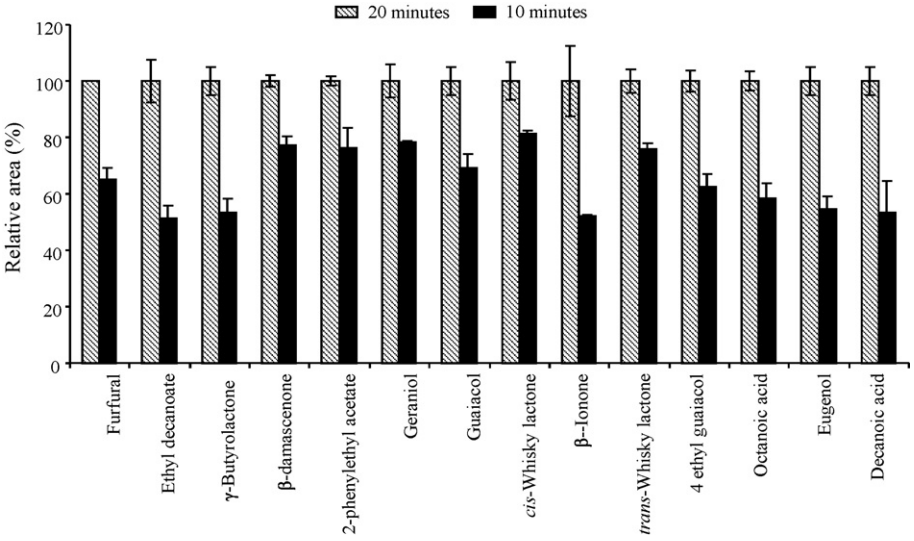

Fig. 1. Comparison of the extraction efficiency of the CAR–PDMS-SPME procedure using two different extraction times.

the individual TIC signal of all the volatiles present in the sample), instead of comparing the individual TIC responses of the volatile compounds, may explain the differences in the results.

3.2. Performance characteristics of the SPME, SPE and LLE methods

The starting point for the comparison of the three extraction methods was in each case previously optimised to give the best recoveries for all the studied volatile compounds. The conditions used for the CAR–PDMS-SPME were set up in the above-mentioned experiment. For SPE, we used a modified extraction procedure based on that described by Lopez et al. [15] while LLE extraction conditions were optimised in our laboratory from a previous method [4] The comparison between the three methodologies was carried out taking into consideration their analytical performance.

3.2.1. Reproducibility

The reproducibility was estimated as the relative standard deviation (RSD) of the areas relative to the internal standard for six extractions of the synthetic wines with the aroma compounds carried out over consecutive days. The RSD for all the volatile compounds using the LLE method ranged between 10% for 2-phenylacetate and 17% for ethyl decanoate, with 12% being the average RSD value for all the volatile compounds. Taking into consideration that there were two consecutive concentration steps after the liquid extraction, these values are quite acceptable. In general, the SPE method showed better reproducibility and the RSD values ranged between 1% for linalool and geraniol and 21% for acetoin. The average RSD values using SPE was 5%, which are similar or even lower to those described by Lopez et al. [15] although they were calculated in real wine samples. In addition, the SPME procedure showed very good reproducibility with RSD values between 2% for ethyl octanoate and 13% for octanoic acid, with an average of 7%.

3.2.2. Linearity

For each compound, a linear regression of the TIC area ratio (TIC volatile compound/TIC internal standard) vs. concentration, was calculated to determine the linearity of the analytical methods, using two replicates at seven levels of concentration for the LLE and SPE procedures and six levels in the case of SPME (therefore, $n = 14$ or 12 points respectively). These concentration levels covered the concentration ranges expected for the aroma compounds found in wine. To judge the adequacy of the linear models, the *F*-ratio for lack of fit was calculated [38]. The regression results and the linear ranges can be found in Tables 1–3.

The linear ranges (Tables 1–3) were in general quite wide for most of the compounds in the three methods. In each case they were between 2 $\mu g\,L^{-1}$ and 5000 or even 10,000 $\mu g\,L^{-1}$. The upper limits of linearity for the SPE and SPME methods were in general lower (Table 2) than those calculated for LLE (Table 1). This seems to show a saturation of the cartridge (in the SPE method) or of the polymeric phase of the fibre (in the SPME method) when the concentration of the analyte was above 5000 $\mu g\,L^{-1}$. The lower limits of linearity were however the same for the three methodologies. In each case, the linear ranges covering a broader range of wine

Table 1
Results of linear regression ($y = a + bx$) for TIC area vs. concentration and analytical performance for the volatile compounds in synthetic wines determined by the LLE procedure.

Compounds	Reproducibility (RSD, %)[a]	Analytical performance[b]						
		Linear range ($\mu g\,L^{-1}$)	b	R^2	s	CV (%)	Detection limit[c] ($\mu g\,L^{-1}$)	Recovery (%)
Isobutyl acetate	11	2–10,000	2.871	0.997	0.628	9.2	1.0	107
Ethyl butanoate	11	2–10,000	3.011	0.998	0.550	7.7	1.0	94
Butyl acetate	11	2–10,000	2.947	0.998	0.590	8.4	1.0	90
Isopentyl acetate	11	2–10,000	3.697	0.997	0.899	10.2	0.8	100
Ethyl hexanoate	12	2–10,000	4.464	0.997	1.098	10.4	0.6	92
Hexyl acetate	12	2–10,000	4.357	0.996	1.211	11.7	0.6	97
Acetoin	13	2–10,000	0.122	0.992	0.048	16.7	24.7	215
1-Hexanol	13	2–10,000	2.622	0.998	0.554	8.9	1.1	95
cis-3-Hexen-1-ol	14	2–10,000	2.258	0.997	0.531	10.0	1.3	106
Ethyl octanoate	14	2–10,000	5.909	0.996	1.591	11.4	0.5	91
Furfural	12	2–10,000	3.612	0.997	0.901	10.5	0.8	52
Linalool	11	2–10,000	5.925	0.996	1.689	12.0	0.5	107
Ethyl decanoate	17	2–10,000	0.6482	0.998	0.131	8.6	4.6	67
γ-Butyrolactone	14	2–10,000	0.089	0.99	0.039	18.5	34.1	105
Isovaleric acid	15	2–10,000	0.902	0.996	0.241	11.5	3.3	122
Diethyl succinate	11	2–10,000	5.918	0.996	1.579	11.3	0.5	112
α-Terpineol	12	2–10,000	6.106	0.996	1.621	11.2	0.4	96
β-Damascenone	10	2–10,000	1.519	0.996	0.411	11.4	2.0	104
2-Phenylethyl acetate	11	2–10,000	3.522	0.996	0.976	11.7	0.8	93
Hexanoic acid	16	2–10,000	0.727	0.997	0.118	6.9	4.1	106
Geraniol	12	2–10,000	1.435	0.996	0.396	11.6	2.1	90
Guaiacol	12	2–10,000	6.238	0.995	1.871	12.7	0.4	96
cis-Whisky lactone	12	2–5,000	3.125	0.995	0.475	14.5	0.9	84
β-Ionone	12	2–10,000	8.845	0.998	1.861	9.0	0.3	94
trans-Whisky lactone	11	2–10,000	3.489	0.998	0.669	8.1	0.8	92
4-Ethyl guaiacol	12	2–5,000	9.859	0.993	1.798	17.5	0.3	92
Octanoic acid	13	2–10,000	4.648	0.997	1.069	9.7	0.6	102
Eugenol	12	2–5,000	9.180	0.993	1.726	18.1	0.3	94
Decanoic acid	13	2–10,000	6.066	0.999	0.709	5.0	0.5	101
Vanillin	12	2–10,000	6.058	0.997	1.46	10.3	0.5	58

[a] The reproducibility was estimated as the relative standard deviation (RSD) of the areas relative to the internal standard for six extractions of the synthetic wines with the aroma compounds over consecutive days.

[b] All regressions presented a value of the parameter a not significantly different from zero ($p > 0.05$); R^2, coefficient of determination; s, residual standard deviation; CV (%) = $(s/\bar{y}) \times 100$, residual standard deviation expressed as a percentage of the mean value; recovery = (amount of compound found in the spiked sample − amount found in the sample) × 100/amount of compound added.

[c] Detection limits were estimated as the volatile concentration which gave a signal equal to the blank signal plus 3 standard deviations of the blank.

Table 2
Results of linear regression ($y = a + bx$) for TIC area vs. concentration and analytical performance for the volatile compounds in synthetic wines determined by the SPE procedure.

Compounds	Reproducibility (RSD, %)[a]	Regression characteristics[b]						
		Linear range ($\mu g\,L^{-1}$)	b	R^2	s	CV (%)	Detection limit[c] ($\mu g\,L^{-1}$)	Recovery (%)
Isobutyl acetate	4	2–5,000	3.630	0.985	0.020	0.4	0.4	97
Ethyl butanoate	4	2–5,000	3.817	0.994	0.695	14.2	0.4	95
Butyl acetate	3	2–5,000	3.970	0.997	0.555	10.8	0.4	82
Isopentyl acetate	4	2–5,000	4.741	0.997	0.597	9.7	0.3	74
Ethyl hexanoate	3	2–5,000	5.343	0.970	0.634	9.2	0.3	85
Hexyl acetate	3	2–5,000	5.549	0.998	0.549	7.6	0.3	81
Acetoin	21	2–10,000	0.038	0.978			46.5	862
1-Hexanol	2	2–5,000	4.810	0.998	0.526	8.4	0.3	94
cis-3-Hexen-1-ol	3	2–5,000	5.038	0.996	0.734	11.3	0.3	80
Ethyl octanoate	3	2–5,000	6.363	0.999	0.517	6.3	0.2	90
Furfural	5	2–5,000	2.400	0.997	0.300	9.6	0.7	19
Linalool	1	2–5,000	8.518	0.998	0.958	8.7	0.2	108
Ethyl decanoate	4	2–10,000	0.546	0.996	0.169	11.4	3.2	99
γ-Butyrolactone	–	–	–	–	–		–	–
Isovaleric acid	7	2–10,000	2.914	0.989	1.455	17.9	0.6	62
Diethyl succinate	4	2–5,000	8.324	0.998	0.804	7.3	0.2	95
α-Terpineol	2	2–5,000	8.757	0.999	0.736	6.5	0.2	85
β-Damascenone	4	2–5,000	2.076	0.999	0.111	4.1	0.8	96
2-Phenylethyl acetate	3	2–5,000	4.985	0.999	0.437	6.7	0.3	83
Hexanoic acid	14	2–10,000	0.840	0.992	0.364	16.2	2.1	125
Geraniol	1	2–5,000	2.072	0.999	0.145	5.3	0.8	87
Guaiacol	3	2–5,000	9.955	0.998	1.009	7.7	0.1	87
cis-Whisky lactone	3	2–5,000	3.986	0.998	0.440	8.5	0.4	86
β-Ionone	6	2–10,000	9.376	0.997	2.561	9.7	0.1	113
trans-Whisky lactone	3	2–5,000	4.906	0.998	0.567	8.9	0.3	85
4-Ethyl guaiacol	3	2–5,000	12.953	0.998	1.207	7.1	0.1	92
Octanoic acid	13	2–10,000	4.183	0.989	2.066	18.4	0.4	117
Eugenol	3	2–5,000	11.803	0.998	1.174	7.6	0.1	90
Decanoic acid	13	2–5,000	5.420	0.995	0.923	13.7	0.3	117
Vanillin	4	2–5,000	9.516	0.999	0.358	2.9	0.1	58

[a] The reproducibility was estimated as the relative standard deviation (RSD) of the areas relative to the internal standard for six extractions of the synthetic wines with the aroma compounds over consecutive days.

[b] All regressions presented a value of the parameter a not significantly different from zero ($p > 0.05$); R^2, coefficient of determination; s, residual standard deviation; $CV\,(\%) = (s/\bar{y}) \times 100$, residual standard deviation expressed as a percentage of the mean value; recovery = (amount of compound found in the spiked sample − amount found in the sample) × 100/amount of compound added.

[c] Detection limits were estimated as the volatile concentration which gave a signal equal to the blank signal plus 3 standard deviations of the blank.

volatile concentrations, were greater than those recently published for most of these compounds by using stir bar sorptive extraction (SBSE) [36]. While LLE was a method suitable for the extraction of all the studied volatile compounds in the synthetic wines, it was however not possible to extract γ-butyrolactone using SPE. This might be due to the weak affinity of the resin for this compound. This is in agreement with Aznar et al. [29] who did not detect γ-butyrolactone in red wines when using SPE with Amberlite XAD-4 resins either. In addition, SPME was not a suitable method for the extraction of acetoin, isovaleric and hexanoic acids and vanillin. With the use of SPME and/or SBSE relatively high detection limits have also been calculated [11,36] for acetoin, and isovaleric and hexanoic acids, demonstrating the difficulties in extracting these compounds from wine samples using these methodologies.

The linearity, as it can be seen with the determination coefficients (Tables 1–3), was excellent when using LLE and SPE methods. In both of them, R^2 was higher than 0.99 for most of the volatile compounds studied. In addition, the residual standard deviation expressed as a percentage of the mean value (CV, %) was lower than 14% for most of the compounds, which shows the adequacy of the regression models. Ferreira et al. [13] also showed very good linearity in a LLE method for 25 wine volatile compounds using 1,1,2-trichlorotrifluoroethane (Freon 113) as a solvent, although they studied a narrower linear range. SPME also showed very good linearity in the range of concentrations studied (Table 3), although R^2 was in general a little bit lower (R^2 above 0.96) and the residual standard deviation expressed as a percentage of the mean value, was in general higher compared to the other two procedures.

The slope of the regression models can be considered as a measurement of the method sensitivity and depends on the extraction efficiency and on the detector response for each compound. In general, very similar slopes were obtained for the LLE and SPE methods. In addition, the same volatile compounds that showed very little slopes by using LLE, such as acetoin, γ-butyrolactone, hexanoic acid and ethyl decanoate, also showed very little slopes using the SPE method. Castro et al. [11] also determined very little sensitivity for acetoin when using continuous liquid–liquid extraction with diethyl ether and n-pentane as the extraction solvents. On the contrary, compounds with higher retention indices, such as 4-ethyl guaiacol, eugenol, decanoic acid, vanillin, etc., showed very high slope values with both methods. The slopes calculated for the volatile compounds using the SPME method, were in general lower that those calculated in the above-mentioned extraction methods, but in agreement with the values published in the literature by using the same fibre coating, although different extraction conditions were used [11]. The differences in sensitivity between different chemical groups were most evident when using SPME than when using the LLE or SPE procedures. In this sense, the esters showed greater sensitivity to the CAR–PDMS fibre, compared to the alcohols or the lactones. The calculated slopes were very similar to those reported by Pozo-Bayon et al. [24] when using a 100 μm PDMS fibre and very different extraction conditions.

3.2.3. Detection limits and accuracy

Detection limits were estimated as the volatile concentration which gave a signal equal to the blank signal plus 3 standard deviations of the blank [39]. As it can be seen in Tables 1 and 2, the

Table 3
Results of linear regression ($y = a + bx$) for TIC area vs. concentration and analytical performance for the volatile compounds in synthetic wines determined by the SPME procedure.

Compounds	Reproducibility (RSD, %)[a]	Regression characteristics[b]						
		Linear range ($\mu g\,L^{-1}$)	b	R^2	s	CV (%)	Detection limit[c] ($\mu g\,L^{-1}$)	Recovery (%)
Isobutyl acetate	5	2–5000	0.060	0.996	0.008	11.3	23.6	112
Ethyl butanoate	9	2–5000	0.100	0.994	0.0167	14.1	14.2	91
Butyl acetate	9	2–1000	0.113	0.987	0.0287	20.7	12.6	118
Isopentyl acetate	7	2–5000	0.158	0.990	0.0401	20.3	9.0	–
Ethyl hexanoate	6	2–1000	1.151	0.980	0.0681	8.2	1.2	55
Hexyl acetate	7	2–1000	1.287	0.910	0.0645	7.2	1.1	92
Acetoin	–	–	–	–	–		–	–
1-Hexanol	5	2–1000	0.036	0.992	0.0017	6.3	39.4	20
cis-3-Hexen-1-ol	8	2–5000	0.013	0.969	0.0052	30.3	107.7	96
Ethyl octanoate	2	2–5000	3.011	0.996	0.4056	11.6	0.4	101
Furfural	7	2–5000	0.048	0.990	0.0105	20.2	29.6	40
Linalool	9	2–1000	0.230	0.990	0.0123	7.7	6.2	110
Ethyl decanoate	7	2–5000	0.489	0.992	0.0947	18.5	2.9	86
γ-Butyrolactone	5	2–5000	0.005	0.988	0.0013	23.9	270.4	86
Isovaleric acid	–	–	–	–	–		–	–
Diethyl succinate	10	2–5000	0.011	0.977	0.004	28.7	121.4	88
α-Terpineol	7	2–5000	0.049	0.985	0.0133	22.5	28.7	114
β-Damascenone	7	2–5000	0.069	0.993	0.0124	16.0	20.7	103
2-Phenylethyl acetate	8	2–5000	0.064	0.992	0.0131	18.6	22.0	79
Hexanoic acid	–	–	–	–	–		–	–
Geraniol	7	2–5000	0.01	0.987	0.0025	24.4	143.3	68
Guaiacol	5	2–5000	0.022	0.987	0.0055	22.8	64.2	97
cis-Whisky lactone	7	2–5000	0.007	0.997	0.0009	11.3	199	108
β-Ionone	6	2–5000	0.324	0.996	0.0446	12.5	4.4	76
trans-Whisky lactone	4	2–5000	0.006	0.985	0.0016	23.3	238.9	104
4-Ethyl guaiacol	5	2–5000	0.033	0.977	0.0111	31.6	43.3	86
Octanoic acid	13	2–5000	0.007	0.984	0.0021	28.0	186.1	116
Eugenol	10	2–5000	0.010	0.986	0.0027	24.7	137.8	92
Decanoic acid	8	2–5000	0.016	0.967	0.007	39.7	86.3	82
Vanillin	–	–	–	–	–		–	–

[a] The reproducibility was estimated as the relative standard deviation (RSD) of the areas relative to the internal standard for six extractions of the synthetic wines with the aroma compounds over consecutive days.

[b] All regressions presented a value of the parameter a not significantly different from zero ($p > 0.05$); R^2, coefficient of determination; s: residual standard deviation; $CV(\%) = (s/\bar{y}) \times 100$ residual standard deviation expressed as a percentage of the mean value; recovery = (amount of compound found in the spiked sample − amount found in the sample) × 100/amount of compound added.

[c] Detection limits were estimated as the volatile concentration which gave a signal equal to the blank signal plus 3 standard deviations of the blank.

LLE and SPE methods gave very similar and in general very low detection limits, the latter showed even lower detection limits. Acetoin gave poor results with both methods and showed considerably high detection limits (24.7 and 46.5 $\mu g\,L^{-1}$) compared to the rest of volatile compounds. By using SPME, it was not possible to extract this compound. Although in our SPME extraction conditions, we could not determine acetoin in the synthetic wines, Guerrero et al. [36] did. However, they determined a very high detection limit for this compound (2098 $\mu g\,L^{-1}$) compared to the rest of volatiles they studied. In general, using LLE and SPE methods, the detection limits calculated for most of the volatile compounds under study were <1 $\mu g\,L^{-1}$. However, SPE showed the lowest detection limits (above 0.1 $\mu g\,L^{-1}$) for some volatile compounds such as guaiacol, β-ionone, 4-ethyl guaiacol, eugenol and vanillin. These values are in general, in agreement to those determined in wines by Lopez et al. [15], using the same type of SPE resins. When comparing the results with our LLE procedure to those using liquid–liquid microextraction with Freon 113 and gas chromatography ion trap mass spectrometry [13], the detection limits determined using LLE were slightly lower. However, when comparing the detection limits calculated in the present study using LLE to the those by Castro et al. [11] who used continuous liquid–liquid extraction using diethyl ether:pentane for volatile compounds in Sherry wines, the detection limits for LLE were in general more than 10 times lower. However, both methodologies were sufficiently sensitive to measure the volatile compounds in wines. SPME showed in general higher detection limits than LLE and SPE for most of the volatile compounds under study, although the values are in agreement with others published in the literature [11,24]. Taking into consideration the small volume of wine and the short extraction time (20 min) used in the present SPME procedure, for some volatile compounds, namely esters, the conditions used in the present work seem to be more adequate than other SPME protocols [11]. However, the detection limits calculated with SPME for most of the volatile compounds in the present study are higher than those recently reported by Caldeira et al. [37] for volatile compounds in whiskeys. Taking into consideration the wide linear range of concentrations they studied (400–92,000 $\mu g\,L^{-1}$), they reported very low values (<19 $\mu g\,L^{-1}$ for 22 volatile compounds). The reason for the higher detection limit values calculated in the present study might be due to the more restrictive criteria for the calculation of the detection limit in the present work compared to Caldeira's study [37].

The accuracy of the three analytical methods was evaluated from the determination of the recovery obtained (mean values) by the addition of known amounts of the standard mixture of volatile compounds (0.5 and 1 $mg\,L^{-1}$ in triplicate) in a commercial wine sample (Tables 1–3). Recoveries near 100% were obtained for most of the studied volatile compounds using the three extraction procedures. However, furfural and vanillin were poorly recovered (52% and 58% respectively) with the LLE and SPE procedures. In addition, LLE showed a low recovery for ethyl decanoate, while isopentyl acetate had a poor recovery when using the SPE procedure (74%). Lopez et al. [15] have shown that the recovery for relatively more polar compounds can be low using Lichrolut-EN resins because of the low solid–liquid distribution coefficients of these volatile compounds in the polymeric resins. Regarding, the results of recovery by using SPME, we observed very low values for polar compounds

such as 1-hexanol (20%), furfural (40%) and geraniol (68%), although other less polar compounds such as ethyl hexanoate were not recovered very well either (55%). The high recovery values calculated for some compounds, such as acetoin by using LLE and SPE procedures, might be related to the low detector response of this compound, which could have induced an underestimation of the amount of this compound in the wine sample given an unrealistic and elevated recovery value.

Based on the analytical performance of the three methodologies under the experimental conditions employed, it can be concluded that the more traditional extraction techniques, such as LLE and SPE were the most suitable procedures for the extraction of 30 representative wine volatile compounds. In addition, their analytical performances were very similar to each other. Nonetheless, other features of these procedures such as the more tedious sampling treatment, and mainly the use of organic solvents when compared to the SPME procedure are the main drawbacks associated to their use.

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