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Ultrasound-assisted extraction of volatile compounds from wine samples: Optimisation of the method

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Abstract

This study describes a liquid–liquid extraction technique for extracting volatile compounds from wine using dichloromethane and ultrasounds. This technique permits the simultaneous extraction of different samples with high reproducibility. After the preliminary tests, several parameters (sample volume, solvent volume and extraction time) were optimised using a factorial design to obtain the most relevant variables. The analytical characteristics were obtained such as calibration graphs, detection limits ranging from 0.0238 mg L^{-1} for 1-pentanol to 0.261 mg L^{-1} for octanoic acid, quantification limits and relative standard deviation from 2.1 to 6.2%. Extraction yields were calculated giving a range 9.16–1.2%. The optimised conditions were applied to the extraction of samples of young wines from the *Denominación de Origen Calificada* Rioja category using gas chromatography and a flame ionisation detector.

Keywords: Volatile compounds; Ultrasound extraction; Wine

1. Introduction

Volatile compounds play an important role in the aroma of wines. Several hundred chemically different compounds (alcohols, esters, acids, terpenes, ...) have been found [1] in wines. The flavour of a wine is very complex, due to the large number of compounds present with different polarities, volatilities and in a wide range of concentrations.

Volatile compound determination in wine includes a sample preparation process, and although the importance [2] of this step has long been recognized, the development and implementation of a new sample preparation process is very slow as compared to other parts of the analytical method such as sampling, identification or quantification.

Gas chromatography is, according to the bibliography, the most used technique for determining volatile compounds. As regards extraction methods, although some authors propose direct injection in the gas chromatograph without prior extraction [3], most studies recommend an extraction step previous to the determination of volatile compounds in wine.

These include liquid–liquid extraction [4–6], purge and trap [7], microextraction [8], solid phase extraction [9–11], supercritical fluid extraction [12,13], pervaporation [14,15] or stir bar sorptive extraction [16].

As revealed by the large number of recent publications, solid phase microextraction (SPME) is gaining popularity as a technique for determining volatile compounds in numerous matrixes [17]. There are many examples that can be cited relating to wine analysis [6,10,18,19]. The main advantages of this method are: it is a solvent-free method; small sample volume, high sensitivity and simplicity. However, a specific and expensive instrumentation is required, that could not be available in all laboratories. In addition, other options should always be considered.

Ultrasound assistance is being used more and more in analytical chemistry, enabling different steps in the analytical process, particularly in sample preparation [20]. Ultrasound-assisted methods have been used to extract toxic elements in contaminated soil samples [21] and in meat sample preparation prior to the determination of metals [22,23], as well as for extract-

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ing metals from seafood [24] or animal feeds [25]. Ultrasounds have also been used in leaching of phenoxyacid herbicides in soil and sediment [26] and in the determination of chemical oxygen demand [27].

Sonication sampling (extraction by ultrasounds) of samples is an alternative technique to the classic liquid–liquid extraction method with organic solvents. Some references according to this procedure are commented below. Thus, the isolation of volatile compounds from citrus flowers and citrus honey [28] was performed using a *n*-pentane:diethylether mixture and a water bath with ultrasound assistance. Sometimes the ultrasound-assisted methodology may not seem to be the best alternative, for example, in the determination of the 2,4,6-trichloroanisole content of tainted cork [29]. But in other cases it is a very good option, such as in the extraction of aroma compounds in aged brandies [30], white wine [31] or must and wine [32].

Our research group has optimised several methodologies for extracting volatile compounds (alcohols, terpenes, acids, esters, ...) from wine based on microextraction with salts [33,34], liquid–liquid extraction (continuous and discontinuous) and solid phase extraction [35] and solid phase microextraction [36]. The present study describes a method based on liquid–liquid extraction by means of ultrasounds. The compounds studied were 3-methyl-1-butyl acetate, 3-methyl-1-butanol, ethyl hexanoate, 1-pentanol, 1-hexanol, ethyl octanoate, linalool, diethyl succinate, α-terpineol, 2-phenyl ethyl acetate, hexanoic acid, 2-phenyl ethyl alcohol, octanoic acid and decanoic acid. The method was applied to young wines from the *Denominación de Origen Calificada* Rioja category.

2. Experimental

2.1. Apparatus

Measurements were taken with a Varian (Walnut Creek, CA, USA) CP-3800 gas chromatograph, equipped with a FID detector, a polar capillary column (HP Innowax 30 m × 0.25 mm i.d. × 0.25 μm film thickness) (Agilent Technologies, Palo Alto, CA, USA) and a CP 8200 injector.

The chromatographic conditions were: injector temperature, 220 °C; detector, 220 °C; for column oven temperature, the following program was used to separate the compounds after extraction: 60 °C (maintained for 4 min) rising to 170 °C at 4 °C min^{-1} (maintained for 12 min). The carrier gas was helium at a flow rate of 0.9 mL min^{-1}; split rate, 0.2:1; flow rates for FID detector: auxiliary gas (He), 30 mL min^{-1}; air, 400 mL min^{-1}; hydrogen, 30 mL min^{-1}. The sample volume injected was 2.0 μL (Hamilton microsyringe 0084857) (Bonaduz, Switzerland).

To identify the compounds present in wine and select some of them, a HP 5989B Mass spectrometer coupled with a HP gas chromatograph 5890 Serie II Plus was used. The instrumental conditions were as follows: interface temperature, 230 °C; electronic impact as the ionisation technique; energy, 70 eV; mass range, 45:700; resolution, 1 atomic mass unit.

The ultrasounds bath was of 5 L of capacity and 40 KHz from J.P. Selecta (Barcelona, Spain).

Statgraphics Plus 4.0 software (Manugistics Inc., Rockville, MA, USA) for experimental design and SPSS 12.0 software (SPSS Inc., Chicago, IL, US) for calibration were used.

2.2. Reagents and solutions

The volatile compounds used (99.0–99.5% purity) were purchased from Fluka (Buchs, Switzerland), Sigma (St. Louis, MO, USA) or Aldrich (Milwaukee, WI, USA). The stock solution was prepared as follows: the compounds were weighed, dissolved in the minimum amount of acetone (Merck: Darmstadt, Germany) and diluted with synthetic wine to a perfectly known volume. The concentration of each compound was exactly known, always around 1000 mg L^{-1} and kept in the refrigerator. Standard solutions were prepared each day by serial dilution of the stock solution.

The solvents used were: hexane (Carlo Erba: Rodano, MI, Italy), methyl isobutyl ketone (Panreac: Barcelona, Spain), petroleum ether (Lab Scan: Dublin, Ireland), pentane (Fluka), dichloromethane (Merck) and diethyl ether (Aldrich).

Solid anhydrous ammonium sulphate, monohydrated sodium dihydrogen phosphate and sodium chloride were purchased from Merck.

The synthetic wine was a hydro-alcoholic solution at 12% (v/v) ethanol where the pH was adjusted to 3.5 with tartaric acid (Merck). Ethanol was purchased from Panreac, and the water was obtained from a Milli-Q apparatus (Millipore: Bedford, MA, USA).

Young red wines from the 2002 vintage were used in this study. The grape variety was not specified, but the four varieties from the *Denominación de Origen Calificada* Rioja are *Tempranillo*, *Garnacha*, *Graciano* and *Mazuelo*.

2.3. Procedure

The procedure applied for extraction and determination was as follow:

Twenty-five millilitres of synthetic wine (described above) containing the volatile compounds (in a concentration around 50.00 mg L^{-1}) or 25.0 mL of wine were placed in a 100 mL beaker together with 10.0 mL of dichloromethane and 4 g of NaCl. The flask was covered using parafilm and a needle was injected to allow air to escape. Then, the solution was extracted by means of ultrasounds for 15 min. Bath temperature was maintained at 25 °C. The solution was then left for a few minutes to separate both phases. The organic phase was separated, 5.0 mL was mixed with the internal standard (3-octanol, concentration around 50.00 mg L^{-1}) and 2.0 μL of this solution was injected into the gas chromatograph. Then, the ratio A_c/A_{is} was experimentally obtained (A_c, area of the compound and A_{is}, area of the internal standard). All the studies were carried out in triplicate and each sample was injected into the gas chromatograph four times. The extracts were stored in a refrigerator until analysis by GC-FID.

2.4. Extraction yield

Extraction yields were calculated using the internal standard method. Standard solutions in dichloromethane containing the volatile compounds and the internal standard at known concentrations were injected into the chromatograph. The response factor (K) of each compound was calculated using the following equation:

$$K = \frac{A_c \times C_{is}}{A_{is} \times C_c}$$

where A_c is area of the compound, A_{is} area of the internal standard, C_c concentration of the compound and C_{is} is the concentration of the internal standard.

Then, 25.0 mL of synthetic wine containing the volatile compounds at known concentrations was subjected to the extraction procedure described above. The concentration of each volatile compound can be calculated by re-applying the response factor equation (K). Taking into account the organic phase volume, the amount of extracted compound was calculated and the yield expressed as (%, w/w) is:

$$\% = \frac{\text{mg extracted}}{\text{mg initial}} \times 100$$

The mean extraction yield values are shown in Table 1.

Table 1
Extraction yields (%)

Compound	%
3-Methyl-1-butyl acetate	38.4
3-Methyl-1-butanol	55.2
Ethyl hexanoate	20.2
1-Pentanol	42.5
1-Hexanol	38.7
Ethyl octanoate	41.4
Linalool	43.2
Diethyl succinate	28.1
α-Terpineol	81.2
2-Phenyl ethyl acetate	39.9
Hexanoic acid	49.8
2-Phenyl ethyl alcohol	32.5
Octanoic acid	9.16
Decanoic acid	23.5

3. Results and discussion

Firstly, the Rioja wine samples were analysed by gas chromatography–mass spectrometry (GC–MS); many compounds were identified, but only 14 were selected for the study, including alcohols, esters, acids and terpenes: 3-methyl-1-butyl acetate, 3-methyl-1-butanol, ethyl hexanoate, 1-pentanol, 1-hexanol, ethyl octanoate, linalool, diethyl succinate, α-terpineol, 2-phenyl ethyl acetate, hexanoic acid, 2-phenyl ethyl alcohol, octanoic acid and decanoic acid.

Five parameters can be considered to be the most relevant in the extraction yield: type of solvent, addition or non-addition of salts (type and concentration), sample volume, solvent volume and ultrasonication time (or extraction time). Ultrasound bath temperature was not considered because some authors have reported no differences in extraction yields when temperature is modified.

3.1. Solvent and salt choice

Initially, a number of prior experiments were performed to establish the most suitable extraction solvent and salt. Both parameters were optimised independently.

Hexane, methyl isobuthyl ketone, petroleum ether, pentane, dichloromethane and ethyl ether were studied as solvent extraction. A synthetic wine solution (25.0 mL) was extracted in accordance with the procedure described above, with 10.0 mL as solvent volume and 20 min as extraction time. No salt was used. In these conditions, the extraction yields were calculated and the results are shown in Fig. 1. As can be seen, hexane is a very bad extraction solvent because only six compounds are extracted and, in the best of cases, around 30% is extracted. When methyl isobuthyl ketone, pentane or ethyl ether was used,

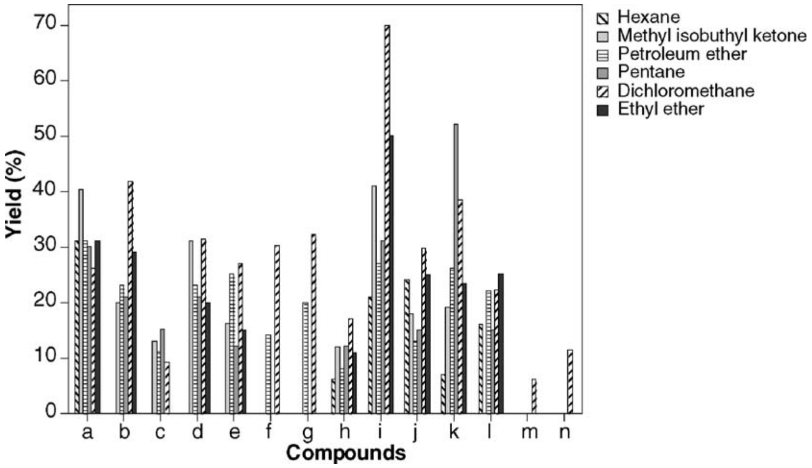

Fig. 1. Extraction yields (%) using different solvents: (a) 3-methyl-1-butyl acetate, (b) 3-methyl-1-butanol, (c) ethyl hexanoate, (d) 1-pentanol, (e) 1-hexanol, (f) ethyl octanoate, (g) linalool, (h) diethyl succinate, (i) α-terpineol, (j) 2-phenyl ethyl acetate, (k) hexanoic acid, (l) 2-phenyl ethyl alcohol, (m) octanoic acid, (n) decanoic acid.

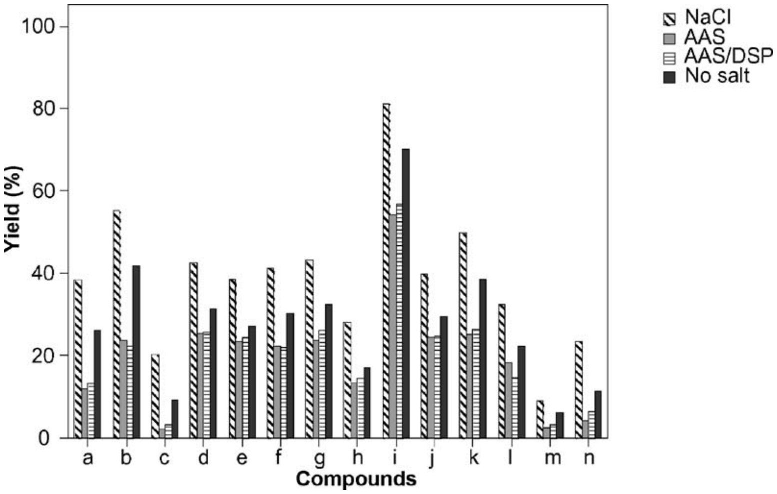

Fig. 2. Extraction yields (%) using different salts: (a) 3-methyl-1-butyl acetate, (b) 3-methyl-1-butanol, (c) ethyl hexanoate, (d) 1-pentanol, (e) 1-hexanol, (f) ethyl octanoate, (g) linalool, (h) diethyl succinate, (i) α-terpineol, (j) 2-phenyl ethyl acetate, (k) hexanoic acid, (l) 2-phenyl ethyl alcohol, (m) octanoic acid, (n) decanoic acid.

four compounds were not extracted: ethyl octanoate, linalool, octanoic acid and decanoic acid. And finally, between petroleum ether and dichloromethane, the best results were obtained with dichloromethane, because with the latter all the compounds were extracted and the yields were bigger in most cases.

As is known, the addition of salts to aqueous media prompts a reduction in the solubility of analytes in the medium in question, thus favouring the extraction process. The equilibrium depends mainly on the type and amount of salt added; hence, we studied the effect of these variables on the extraction yields. Firstly, a number of analyses were performed using different quantities of NaCl (0, 1, 2, 3, 4 and 5 g); the other conditions were maintained (25.0 mL of synthetic wine, 10.0 mL of dichloromethane and 20 min as extraction time). These initial results showed that 4 g was the best option. Quantities below 4 g afforded yields below 55% in all cases. For 4 g yields were between 9% (for octanoic acid) and 81% (for α-terpineol). When 5 g were used, the percentage values were similar to those obtained using 4 g.

The total amount of salt was 4 g but the type of salt was different. Thus, anhydrous ammonium sulphate (AAS) and dihydrate sodium phosphate (DSP) were used. A 2 g AAS/2g DSP mixture was also used because, according to our experience [33], it could be a good option. The results obtained are shown in Fig. 2. All the compounds were extracted in all cases, but the best results were obtained with 4 g of sodium chloride.

3.2. *Factorial design*

Once dichloromethane and 4 g of NaCl had been chosen as the optimum parameters, other variables affecting the extraction procedure, such as sample volume, solvent volume and extraction time, were optimised using a 2^3 factorial design with two centrals points and two levels for each factor. All the experiments were performed in duplicate and randomised. Table 2 shows the factors and the corresponding levels. Statgraphics was used and the experimental design matrix is shown in Table 3. The response considered is the ratio A_c/A_{is} (A_c, area of the compound and A_{is}, area of the internal standard).

Table 2
Variables and levels considered in the factorial design

Variables	Level +	Level −
Sample volume, x_1 (mL)	50.0	25.0
Solvent volume, x_2 (mL)	10.0	5.00
Extraction time, x_3 (min)	30	15

After processing the data using Statgraphics software, the ANOVA tables were constructed to test the significance of the effects. The strength of the influence of a factor is indicated by the magnitude of the *F*-value (factors with *F*-values over 5.318 have a significant influence at the 5% significance level), while the direction of this influence is shows by the sign of the effect. Table 4 shows the significance and the direction of the effect of the factors. It can be seen that for all compounds, the significant variables were solvent volume (x_2), extraction time (x_3) and interaction between the sample volume and solvent volume (x_1x_2). Sample volume (x_1) and interaction between solvent volume and extraction time (x_2x_3) were significant in most cases.

Table 3
Experimental matrix ($2^3 + 2$)

N	Codified variables			No codified variables		
	x_1	x_2	x_3	x_1	x_2	x_3
1–12	0	0	0	37.5	7.50	22.5
2–18	+	−	−	50.0	5.00	15
3–19	−	+	+	25.0	10.0	30
4–13	+	+	−	50.0	10.0	15
5–16	−	−	−	25.0	5.00	15
6–20	−	+	−	25.0	10.0	15
7–14	+	−	+	50.0	5.00	30
8–15	−	−	+	25.0	5.00	30
9–11	+	+	+	50.0	10.0	30
10–17	0	0	0	37.5	7.50	22.5

x_1: sample volume (mL); x_2: solvent volume (mL); x_3: extraction time (min).

Table 4
Results obtained in the factorial design: significance and direction of the factors

	x_1	x_2	x_3	x_1x_2	x_2x_3	x_1x_3
3-Methyl-1-butyl acetate	NS	S(+)	S(−)	S(−)	S(−)	NS
3-Methyl-1-butanol	NS	S(+)	S(−)	S(−)	S(−)	NS
Ethyl hexanoate	S(+)	S(+)	S(−)	S(−)	S(−)	NS
1-Pentanol	S(−)	S(+)	S(−)	S(−)	NS	NS
1-Hexanol	NS	S(+)	S(−)	S(−)	S(−)	NS
Ethyl octanoate	S(−)	S(+)	S(−)	S(−)	S(−)	NS
Linalool	S(−)	S(+)	S(+)	S(−)	NS	NS
Diethyl succinate	S(−)	S(+)	S(−)	S(−)	NS	NS
α-Terpineol	S(−)	S(+)	S(−)	S(−)	S(+)	NS
2-Phenyl ethyl acetate	S(−)	S(+)	S(−)	S(−)	S(+)	NS
Hexanoic acid	S(−)	S(+)	S(−)	S(−)	S(−)	NS
2-Phenyl ethyl alcohol	S(−)	S(+)	S(−)	S(−)	S(−)	S(+)
Octanoic acid	S(−)	S(+)	S(−)	S(−)	NS	NS
Decanoic acid	S(−)	S(+)	S(+)	S(−)	NS	NS

S: significative; NS: no significative. x_1: sample volume (mL); x_2: solvent volume (mL); x_3: extraction time (min).

Table 5
Optimum parameters

Parameters	
Solvent	Dichloromethane
Salt	NaCl, 4 g
Sample volume	25.0 mL
Solvent volume	10.0 mL
Extraction time	15 min
Detector temperature	220 °C
Column	HP Innowax
Injector temperature	220 °C
Flow gas	Helium, 0.9 mL min^{-1}
Auxiliary gas	Helium, 30 mL min^{-1}
Air	400 mL min^{-1}
Hydrogen	30 mL min^{-1}
Split ratio	0.2:1
Injection volume	2.0 μL
Oven temperature	$\underset{4\ \mathrm{min}}{60\,^{\circ}\mathrm{C}} \xrightarrow{4\,^{\circ}\mathrm{C\,min}^{-1}} \underset{12\ \mathrm{min}}{170\,^{\circ}\mathrm{C}}$

Lastly, interaction between sample volume and extraction time (x_1x_3) was not significant, except in the case of 2-phenyl ethyl alcohol.

As regards the direction of the effects, indicated by the sign (positive for the highest level and negative for the lowest level), it can be seen that sample volume (x_1) was affected by a negative sign in all significant variables, except in the case of ethyl hexanoate, so 25.0 mL of sample volume is better than 50.0 mL; solvent volume (x_2) had a positive sign, so 10.0 mL of solvent volume is better than 5.00 mL; extraction time (x_3) was negative, except in the case of linalool and decanoic acid, so 15 min of extraction time is better than 30 min; interaction x_1x_2 was negative and interaction x_2x_3 was also mainly negative.

Since the experiments were performed in duplicate (there were two blocks of experiments), a comparative analysis between blocks was carried out to calculate the so-called "block effect". The conclusion was that there were no significant differences between the replicated blocks.

The equation obtained for the model was:

$$y = \beta_0 + \beta_1x_1 + \beta_2x_2 + \beta_3x_3 + \beta_{12}x_1x_2 + \beta_{23}x_2x_3$$

where y is the predicted response, x_i the factors and β_i are the adjusted coefficients.

Based on these results and previous experiments, the conditions considered as optimum are shown in Table 5 and the chromatogram obtained in these conditions is shown in Fig. 3.

3.3. Analytical characteristics

The analytical characteristics were obtained in the optimum conditions described in Table 5. The calibration graphs were constructed with three replicates of six standard solutions prepared in synthetic wine using 3-octanol as internal standard (50.00 mg L^{-1}). First, a linearity study was made and in Table 6

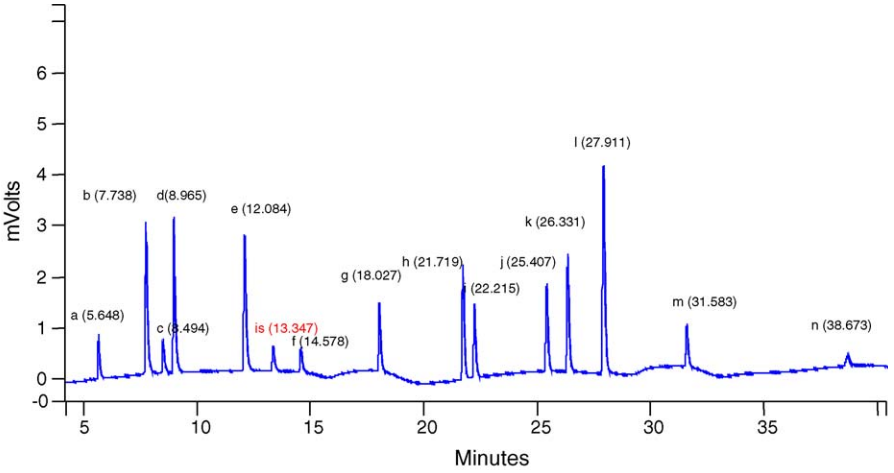

Fig. 3. Chromatogram obtained using the optimum conditions: (a) 3-methyl-1-butyl acetate, (b) 3-methyl-1-butanol, (c) ethyl hexanoate, (d) 1-pentanol, (e) 1-hexanol, (f) ethyl octanoate, (g) linalool, (h) diethyl succinate, (i) α-terpineol, (j) 2-phenyl ethyl acetate, (k) hexanoic acid, (l) 2-phenyl ethyl alcohol, (m) octanoic acid, (n) decanoic acid. is represents internal standard: 3-octanol.

Table 6
Analytical characteristics

Compounds	HL	Slope (L mg^{-1})	Intercept	DL	QL	S_r (%)
3-Methyl-1-butyl acetate	300	0.00651 ± 0.00012	−0.208 ± 0.013	0.0781	0.261	5.4
3-Methyl-1-butanol	750	0.0171 ± 0.0004	−0.226 ± 0.014	0.0295	0.0980	5.7
Ethyl hexanoate	220	0.00242 ± 0.00004	−0.0681 ± 0.51E−2	0.207	0.691	4.5
1-Pentanol	100	0.00782 ± 0.00017	−0.0872 ± 0.0031	0.0238	0.0791	2.8
1-Hexanol	240	0.0102 ± 0.0001	−0.372 ± 0.094	0.0517	0.173	4.2
Ethyl octanoate	200	0.0100 ± 0.0001	−0.177 ± 0.010	0.0508	0.168	2.1
Linalool	110	0.0168 ± 0.0003	−0.245 ± 0.033	0.0255	0.0847	2.6
Diethyl succinate	220	0.0157 ± 0.0001	4.02E−3 ± 1.49E−4	0.0343	0.110	6.2
α-Terpineol	100	0.0195 ± 0.0031	0.0310 ± 0.41E−2	0.0246	0.0820	2.9
2-Phenyl ethyl acetate	110	0.117 ± 0.001	0.731 ± 0.024	0.0431	0.141	5.3
Hexanoic acid	170	0.00647 ± 0.00009	0.995 ± 0.078	0.0581	0.190	3.6
2-Phenyl ethyl alcohol	210	0.00390 ± 0.00006	1.09 ± 0.02	0.130	0.431	5.1
Octanoic acid	150	0.00194 ± 0.00004	0.441 ± 0.052	0.261	0.867	4.1
Decanoic acid	250	0.00567 ± 0.00007	0.196 ± 0.054	0.0897	0.297	3.4

HL, higher limit of linear range (mg L^{-1}); DL, detection limit (mg L^{-1}); QL, quantification limit (mg L^{-1}); S_r, relative standard deviation.

Table 7
Volatile compounds concentration (mg L^{-1}) found in real wine samples

Compounds	Sample 1	Sample 2	Sample 3	Sample 4
3-Methyl-1-butyl acetate	3.68 ± 1.10	3.08 ± 1.00	3.52 ± 1.14	4.01 ± 1.11
3-Methyl-1-butanol	168 ± 26	152 ± 22	176 ± 18	143 ± 23
Ethyl hexanoate	0.821 ± 0.010	0.754 ± 0.001	1.181 ± 0.101	0.981 ± 0.121
1-Pentanol	0.422 ± 0.001	0.502 ± 0.011	0.431 ± 0.012	0.512 ± 0.022
1-Hexanol	12.0 ± 1.1	11.2 ± 1.1	11.9 ± 1.1	10.2 ± 1.0
Ethyl octanoate	3.71 ± 0.02	2.81 ± 0.12	3.34 ± 0.01	2.91 ± 0.11
Linalool	nd	nd	nd	nd
Diethyl succinate	1.61 ± 0.21	1.52 ± 0.12	1.66 ± 0.11	1.45 ± 0.21
α-Terpineol	nd	nd	nd	nd
2-Phenyl ethyl acetate	5.10 ± 1.31	5.01 ± 1.11	4.91 ± 1.21	4.63 ± 1.02
Hexanoic acid	1.22 ± 0.60	1.71 ± 0.12	1.32v± 0.11	1.53 ± 0.10
2-Phenyl ethyl alcohol	9.42 ± 0.22	8.91 ± 0.23	9.12 ± 0.01	9.32 ± 0.12
Octanoic acid	1.41 ± 0.12	1.14 ± 0.12	1.21 ± 0.13	1.02 ± 0.33
Decanoic acid	0.601 ± 0.010	0.611 ± 0.010	0.632 ± 0.022	0.622 ± 0.022

nd: no detected.

the higher limit of the linear response range is shown for each compound. However, for quantification smaller ranges were employed.

Slope and intercept values were calculated by the linear least-squares method. In all cases, a good correlation ($r > 0.99$) was observed. Detection limits were calculated as a signal three times the height of the blank measurement background. Quantification limits were calculated as a signal 10 times the height of the blank measurement background. Precision was expressed as the relative standard deviation. All of these analytical characteristics are shown in Table 6.

3.4. *Application to wine samples*

Four wine samples from the *Denominación de Origen Calificada* Rioja were analysed in triplicate in accordance with the procedure described. Quantification was performed using calibration graphs and the results are shown in Table 7. Linalool and α-terpineol were not detected.

4. Conclusions

Ultrasound-assisted extraction followed by gas chromatography and flame ionisation detector is a good procedure for the analysis of volatile compounds from samples of red wine. The main advantages of extraction by ultrasounds compared to other conventional methods are that it is simple, rapid and no specific instrumentation is required. Better extraction yields could be obtained with solid phase microextraction, but in our opinion, it is an interesting alternative for extracting volatile compounds.

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References

[1] H.D. Belitz, W. Grosch, Química de los alimentos, second ed., Editorial Acribia, SA, Zaragoza (Spain), 1997.

[2] K. Jinno, Anal. Bioanal. Chem. 373 (2002) 1.
[3] J. Villén, F.J. Señoráns, G. Reglero, M. Herraiz, J. Agric. Food Chem. 43 (1995) 717.
[4] V. Aubry, P.X. Etiévant, C. Giniès, R. Henry, J. Agric. Food Chem. 45 (1997) 2120.
[5] M. Ortega-Heras, M.L. González-SanJosé, S. Beltrán, Anal. Chim. Acta 458 (2002) 85.
[6] R. Castro, R. Natera, P. Benitez, C.G. Barroso, Anal. Chim. Acta 513 (2004) 141.
[7] C. García-Jares, S. García-Martín, R. Cela-Torrijos, J. Agric. Food Chem. 43 (1995) 764.
[8] V. Ferreira, M. Sharman, J.F. Cacho, J. Dennis, J. Chromatogr. A 731 (1996) 247.
[9] K. Wada, T. Shibamoto, J. Agric. Food Chem. 45 (1997) 4362.
[10] S. Insa, V. Salvadó, E. Anticó, J. Chromatogr. A 1047 (2004) 15.
[11] D. Matejícek, O. Mikes, B. Klejdus, D. Sterbová, V. Kubán, Food Chem. 90 (2005) 791.
[12] G.P. Blanch, G. Reglero, M. Herraiz, J. Agric. Food Chem. 43 (1995) 1251.
[13] P. Karásek, J. Planeta, E.V. Ostrá, M. Mikesová, J. Goliás, M. Roth, J. Vejrosta, J. Chromatogr. A 1002 (2003) 13.
[14] J. González-Rodríguez, P. Pérez-Juan, M.D. Luque de Castro, Talanta 59 (2003) 691.
[15] J.L. Gómez-Ariza, T. García-Barrera, F. Lorenzo, J. Chromatogr. A 1049 (2004) 147.
[16] J. Díez, C. Domínguez, D.A. Guillén, R. Veas, C.G. Barroso, J. Chromatogr. A 1025 (2004) 263.
[17] H. Kataoka, H.L. Lord, J. Pawliszyn, J. Chromatogr. A 880 (2000) 35.
[18] J.C.R. Demyttenaere, C. Dagher, P. Sandra, S. Kallithraka, R. Verhé, N. De Kimpe, J. Chromatogr. A 985 (2003) 233.
[19] M. Bonino, R. Schellino, C. Rizzi, R. Aigotti, C. Delfini, C. Baiocchi, Food Chem. 80 (2003) 125.
[20] F. Priego-Capote, M.D. Luque de Castro, Trac-Trend Anal. Chem. 23 (2004) 644.
[21] A. Väisänen, R. Suontamo, J. Silvonen, J. Rintala, Anal. Bioanal. Chem. 373 (2002) 93.
[22] R.M. García-Rey, R. Quiles-Zafra, M.D. Luque de Castro, Anal. Bioanal. Chem. 377 (2003) 316.
[23] A. Moreno-Cid, M.C. Yebra, S. Cancela, R.M. Cespón, Anal. Bioanal. Chem. 377 (2003) 730.
[24] A. Moreno-Cid, M.C. Yebra, Anal. Bioanal. Chem. 379 (2004) 77.
[25] F. Priego-Capote, M.D. Luque de Castro, Anal. Bioanal. Chem. 378 (2004) 1376.
[26] A. Caballo-López, M.D. Luque de Castro, Chromatographia 58 (2003) 257.
[27] A. Canals, M.R. Hernández, Anal. Bioanal. Chem. 374 (2002) 1132.
[28] E. Alissandrakis, D. Daferera, P.A. Tarantilis, M. Polissiou, P.C. Harizanis, Food Chem. 82 (2003) 575.
[29] R. Juanola, D. Subirá, V. Salvadó, J.A. García Regueiro, E. Anticó, J. Chromatogr. A 953 (2002) 207.
[30] I. Caldeira, R. Pereira, M.C. Clímaco, A.P. Belchior, R. Bruno de Sousa, Anal. Chim. Acta 513 (2004) 125.
[31] D. Hernanz Vila, F.J. Heredia Mira, R. Beltrán Lucena, M.A. Fernández Recamales, Talanta 50 (1999) 413.
[32] C. Cocito, G. Gaetano, C. Delfini, Food Chem. 52 (1995) 311.
[33] C. Sáenz-Barrio, T. Cedrón-Fernández, Chromatographia 51 (2000) 221.
[34] T. Cedrón-Fernández, C. Sáenz-Barrio, S. Cabredo-Pinillos, I. Sanz-Vicente, Talanta 57 (2002) 555.
[35] S. Cabredo-Pinillos, T. Cedrón-Fernández, C. Sáenz-Barrio, Anal. Lett. 37 (2004) 3063.
[36] S. Cabredo-Pinillos, T. Cedrón-Fernández, A. Parra-Manzanares, C. Sáenz-Barrio, Chromatographia 59 (2004) 733.