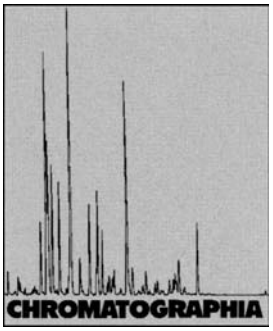
Determination of Volatile Compounds in Wine by Automated Solid-Phase Microextraction and Gas Chromatography



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Abstract

Solid-phase microextraction in headspace mode has been applied to the determination of volatile compounds (3-methyl-1-butyl acetate, 3-methyl-1-butanol, ethyl hexanoate, pentanol, hexanol, linalool, diethyl succinate, α-terpineol, 2-phenylethyl acetate, geraniol, 2-phenylethanol and octanoic acid) in wine samples from the *Denominación de Origen Calificada* Rioja using gas chromatography and a flame ionization detector. After the preliminary tests, several parameters were optimized using a Plackett-Burman design to get the most relevant variables. These parameters were: extraction time, desorption time, split ratio, magnetic stirring, type of fibre, type of injection (headspace or direct sampling) and type of salt. Five wine samples were analysed under optimum conditions. Concentrations ranging from 0.0104 mg L^{-1} for pentanol and 48.9 mg L^{-1} for 3-methyl-1-butanol were obtained. Linalool, α-terpineol and geraniol were not detected. Limit of detection ranging 0.00150-0.00800 mg L^{-1} and relative standard deviation ranging 1.1–5.7% were obtained.

Keywords

Gas chromatography
Head space solid-phase microextraction
Volatile compounds in Rioja wine

Introduction

More than 1000 volatile compounds have been identified in wine samples although only a few are considered responsible for the flavour of wine [1]. Flavour compounds can be classified in three categories: primary, secondary and tertiary. Primary compounds come from the grapes, while secondary compounds are formed during the fermentation process and, finally, tertiary compounds are produced during the aging stage. Flavour-responsible compounds are usually esters, alcohols, organic acids, aldehydes, ketones and other [2–4]. For example, the esters give the fruits flavours (pineapple, banana, peach) or the 6-C compounds impart the herbal notes.

Volatile compounds have an impact on the quality of wine [5], and thus it is necessary to develop analytical determination methods. According to the bibliography, gas chromatography is the most common method to determine volatile compounds in a number of products, such as apple [6], coffee [7], medicinal plants [8, 9] or soil samples [10], and of course, wine is one of them [11, 12]. Nevertheless, a preconcentration and extraction step is usually necessary before the analysis by gas chromatography, due to the complexity of the sample and low concentration of several compounds. For wine, some preparation techniques used are liquid-liquid [13] or solid-phase extraction [14], microextraction [15], ultrasound [16] and others. However, since all these methods have some disadvantages, some new techniques, such as solid-phase microextraction (SPME) are being developed.

Since Pawliszyn et al. [17], described SPME in the early 90, this technique has been widely developed, both in theoretical and instrumental terms [18] and in terms of application [19], as these two reviews prove. Novel fibers have been developed, i.e, to the analysis of aliphatic alcohols and amines [20–22]. There are many references using SPME to analyze volatile compounds in wine [23, 24]. The main advantages of SPME are its high sensitivity (due to the preconcentration and extraction process), the elimination of the solvent, the limited amount of sample necessary and its simplicity. Besides, SPME allows to analyze solid, liquid and gaseous samples and can be combined with different analytical instruments.

The aim of this work was to apply the SPME-GC to develop a method to

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determine twelve volatile compounds (*bouquet* responsible) present in wine (3-methyl-1-butyl acetate, 3-methyl-1-butanol, ethyl hexanoate, pentanol, hexanol, linalool, diethyl succinate, α-terpineol, 2-phenylethyl acetate, geraniol, 2-phenylethanol and octanoic acid). These compounds had been previously analyzed by gas chromatography using other extraction techniques such as microextraction [15, 25], liquid-liquid or solid-phase extraction, and SPME is the best of them. The parameters were optimised from an experimental design approach in order to reduce the number of experiments. The preconcentration factor is used to compare the results. The method was applied to wine samples from La Rioja (Spain).

Experimental

Instrumentation

The analyses were performed on a Varian (Walnut Creek, CA, USA) CP-3800 gas chromatograph equipped with a flame ionization detector (FID) and a capillary column HP-INNOWax (Agilent Technologies, Palo Alto, CA, USA) (30 m × 0.25 mm I.D., 0.25 μm). Helium was used as carrier gas and the detector was fed with synthetic air, hydrogen and helium as auxiliary gas.

An automatic Varian CP-8200 injector was used. The fibres and the SPME syringe accessory were purchased from Supelco (Bellafonte, PA, USA) and four different models were tested: PDMS 100 (coated with 100 μm of polydimethylsiloxane, red colour), CWAX/DVB (coated with 65 μm of carbowax®/divinylbenzene, orange colour), PDMS 7 (coated with 7 μm of polydimethylsiloxane, green colour) and PACR (coated with 85 μm of polyacrylate, white colour). The fibres were conditioned according to the instructions of the manufacturer before use.

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

Statgraphics Plus 4.0 software (Manugistics, Inc., Rockville, MA, USA) for experimental design and SPSS 9.0 software (SPSS Inc., Chicago, IL, USA) for calibration were used.

Reagents

3-methyl-1-butyl acetate, ethyl hexanoate, linalool, α-terpineol, and geraniol were purchased from Aldrich (Milwaukee, WI, USA); 3-methyl-1-butanol, pentanol, diethyl succinate, 2-phenylethyl acetate and 2-phenylethanol were purchased from Fluka (Buschs, Switzerland); hexanol and octanoic acid were purchased from Sigma (St Louis, MO, USA). Sodium chloride, ammonium sulphate, sodium sulphate, potassium sulphate and sodium dihydrogen phosphate were purchased from Merck.

The purity of the volatile compounds was, in all cases, above 99%. Stock solutions were prepared in two different ways: i) in dichloromethane (Merck) and, ii) in water (Milli-Q from Millipore, Bedford, MA, USA), although the compounds were solved in acetone (Merck). The concentration of these stock solutions was approx. 1000 mg L^{-1} and were kept in the refrigerator. Standard solutions were prepared each day by serial dilution of the stock solution.

A synthetic wine was prepared by dissolving stock solutions (in water) of the volatile compounds under study in aqueous ethanol (12% ethanol, Panreac, Barcelona, Spain); the pH was then adjusted to 3.5 with tartaric acid (Merck).

The red wines investigated were young Rioja (Spain) wines of the year 2001.

Procedure

The conditions of the gas chromatograph were as follow: 220 °C as injector and detector temperature, the column (HP-INNOWax) was held at 60 °C for 4 min and then the temperature was increased at a rate of 4 °C min^{-1} up to 170 °C for 12 min; helium at 0.9 mL min^{-1} was used as carrier gas, the flow rates for the FID were 25 mL min^{-1} for the auxiliary gas (He), 30 mL min^{-1} for hydrogen, and 300 mL min^{-1} for air with a split ratio of 0.15:1.

The SPME conditions were as follows: a CWAX/DVB fibre (coated with 65 μm of carbowax®/divinylbenzene) was installed in the SPME syringe accessory and the vials containing 0.800 mL were placed in the automatic injector. The headspace (HS) mode was used and the absorption and desorption times were 10 and 2 min, respectively. Magnetic stirring with no heating was used during the extraction procedure. All solutions were injected, at least, three times using one injection per vial, thus three different vials were needed for each solution.

The procedure applied for the extraction was as follows: 10.0 mL of synthetic wine (described above) containing the volatile compounds were placed into a tube of approx. 20 mL. The internal standard (3-octanol at 15.8 mg L^{-1}) was added and also 0.1 g each of sodium chloride and ammonium sulphate. Finally, the solution was shaken and a volume of 0.800 mL was placed into a capped vial in the automatic injector of the chromatograph. The same procedure was used for the quantification of real samples, except the addition of volatile compounds.

Results and Discussion

Preconcentration Factor

In order to evaluate this extraction technique and compare the results using different conditions, the preconcentration factors were calculated. Solutions containing the volatile compounds (included 3-octanol as internal standard) were prepared in dichloromethane and injected into the gas chromatograph under optimum conditions. Using the internal standard method, the response factor (K) of each compound was calculated following the next equation:

$$K = \frac{A_{vc} \times C_{is}}{A_{is} \times C_{vc}}$$

where A_{vc} is the signal of each compound (peak area or height), A_{is} is the signal of the internal standard, C_{vc} is the concentration of each compound and C_{is} is the concentration of the internal standard.

On the other hand, synthetic wine samples containing the volatile compounds at known concentrations (c_1) were subject to the extraction procedure, and the concentrations were obtained (c_2) using the above mentioned K values. Preconcentration factors were considered as c_1/c_2

Preliminary Tests

Before optimizing any method it is necessary to perform some preliminary tests to assess the behaviour of the system. In this particular case, several tests were performed about the kind of extraction mode, split ratio, absorption time, kind of fibre and number of injections per vial.

Two different extraction modes could be applied, namely introducing the fibre into the synthetic wine sample containing the volatile compounds (direct sampling) or keeping the fibre over the solution without contact (headspace). The experimental conditions are as follow: extraction time 10 min, desorption time 5 min, split ratio 0.15:1, magnetic stirring, CWAX/DVB fibre, sodium chloride and ammonium sulphate at 1% (*w*/*v*), injector and detector temperature 220 °C, the HP-INNOWax column and the temperature program 60 °C for 5 min and then the temperature was increased at 4 °C min^{-1} up to 170 for 9 min. Both modes were compared and the preconcentration factor for each compound was calculated. Table 1 shows the results obtained in three determinations. As it can be seen, no significant differences between both methods were identified, and thus the second option was used in the rest of the work, as the useful life of the fibre was longer.

The samples were injected with a split/splitless injector working on split mode because the splitless one gave an excessive solvent signal. Indeed, if split ratios above 0.15:1 are used some peak could be overlapped with the solvent signal. This parameter was then optimized.

The extraction time (or absorption time) was optimized, but some preliminary tests were performed in order to determine the range. Although very large ranges can be found in the literature (from 5 min-4 h), our experiments proved that extraction times above 10 min do not improve the preconcentration factor.

Another parameter studied was the number of injections per vial (number of injections per vial). It was observed that the signal decreased with the number of injections per vial. An example can be seen in Table 2. In the second injection, losses ranging from 8 to 69% were observed, and in the third, the losses ranged from 60 to 92%. Moreover, the signal was completely lost for 3-methyl-1-buthyl acetate. This parameter was optimized although its influence was clear.

As for the kind of fibre, four different fibres were tested: PDMS 100, CWAX/DVB, PDMS 7 and PACR (the composition of each fibre can be found in the section on Instrumentation). The experimental conditions are as follow: headspace mode, extraction time 10 min, desorption time 2 min, split ratio 0.15:1, magnetic stirring, sodium chloride and ammonium sulphate at 1% (*w*/*v*), injector and detector temperature 220 °C, the HP-INNOWax column and the temperature program 60 °C for 4 min and then the temperature was increased at 4 °C min^{-1} up to 170 for 12 min. The affinity for

Table 1. Preconcentration factors for direct sampling and headspace modes

Compound	Preconcentration Factor	
	Direct sampling	Headspace
3-methyl-1-butyl acetate	0.610 ± 0.050	0.590 ± 0.040
3-methyl-1-butanol	2.39 ± 0.00	2.41 ± 0.11
ethyl hexanoate	5.40 ± 0.17	5.23 ± 0.17
pentanol	4.36 ± 0.14	4.66 ± 0.14
hexanol	2.39 ± 0.23	2.76 ± 0.23
linalool	3.95 ± 0.29	4.42 ± 0.30
diethyl succinate	1.80 ± 0.03	1.82 ± 0.03
α-terpineol	2.94 ± 0.20	2.83 ± 0.19
2-phenylethyl acetate	4.20 ± 0.28	3.81 ± 0.26
geraniol	9.86 ± 0.63	7.18 ± 0.60
2-phenylethanol	3.23 ± 0.13	3.23 ± 0.13
octanoic acid	15.5 ± 0.7	17.7 ± 0.8

$n = 3$

Table 2. Preconcentration factors for different injections per vial

Compound	Preconcentration Factor		
	First injection	Second injection	Third injection
3-methyl-1-butyl acetate	0.591 ± 0.041	0.500 ± 0.062	Nd
3-methyl-1-butanol	2.41 ± 0.11	2.11 ± 0.20	0.516 ± 0.213
ethyl hexanoate	5.23 ± 0.17	4.76 ± 0.23	0.631 ± 0.22
pentanol	4.66 ± 0.23	4.66 ± 0.26	1.32 ± 0.28
hexanol	2.76 ± 0.30	2.52 ± 0.32	0.557 ± 0.36
linalool	4.42 ± 0.03	3.27 ± 0.05	1.22 ± 0.11
diethyl succinate	1.82 ± 0.03	0.835 ± 0.042	0.717 ± 0.06
α-terpineol	2.83 ± 0.19	1.97 ± 0.21	2.83 ± 0.23
2-phenylethyl acetate	3.81 ± 0.26	2.53 ± 0.28	0.623 ± 0.31
geraniol	7.18 ± 0.60	5.63 ± 0.71	0.382 ± 0.72
2-phenylethanol	3.23 ± 0.13	1.28 ± 0.18	0.634 ± 0.22
octanoic acid	17.7 ± 0.8	5.42 ± 0.79	1.32 ± 0.82

$n = 3$
Nd = No detected

Table 3. Factors and values considered in the Plackett-Burman design

x_i	Factor	Level +	Level −
x_1	Extraction time (min)	10	5
x_2	Desorption time (min)	5	2
x_3	Split ratio	0.15:1	0.05:1
x_4	Magnetic stirring	Yes	No
x_5	Fibre	PDMS 100	CWAX/DVB
x_6	Injection per vial	3	1
x_7	*NaCl	Yes	No
x_8	*Na_2SO_4	Yes	No
x_9	*K_2SO_4	Yes	No
x_{10}	*$(NH_4)_2SO_4$	Yes	No
x_{11}	*NaH_2PO_4	Yes	No

* The concentration of the salts is 1% (*w*/*v*)

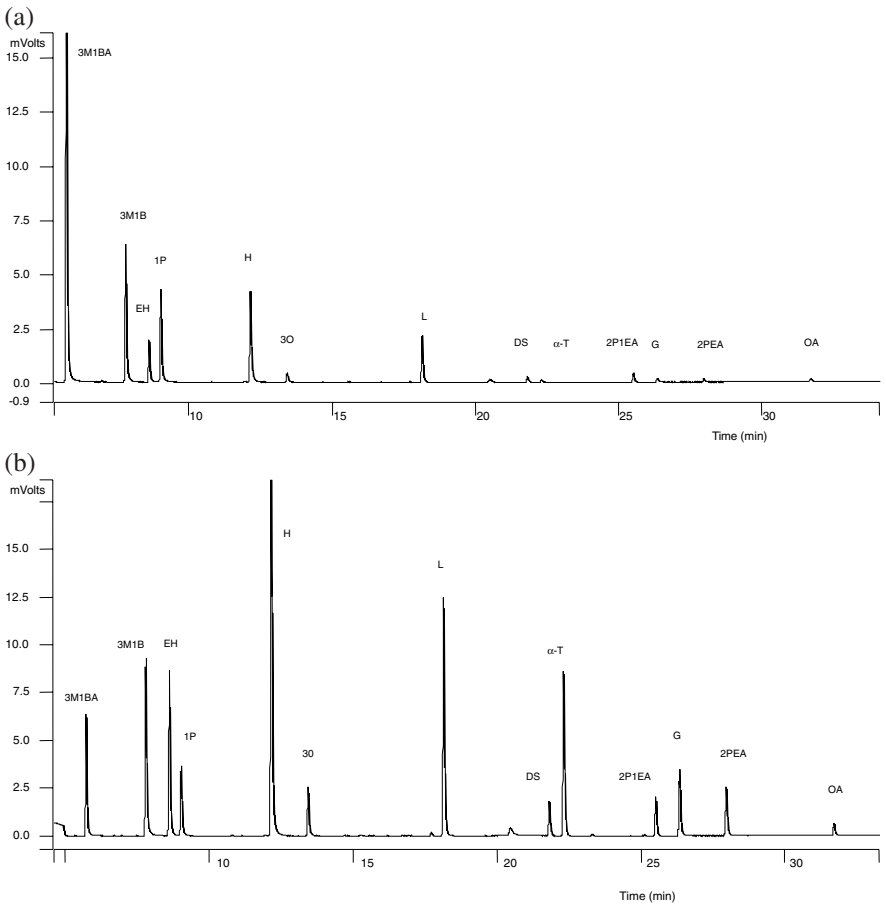

Fig. 1. Chromatograms obtained with synthetic wine using: (a) the PDMS 100 fibre (coated with 100 μm of polydimethylsiloxane) and (b) the CWAX/DVB (coated with 65 μm of carbowax®/divinylbenzene). (**3M1BA** = 3-methyl-1-butyl acetate, **3M1B** = 3-methyl-1-butanol, **EH** = ethyl hexanoate, **1P** = pentanol, **H** = hexanol, **L** = linalool, **DS** = diethyl succinate, α-**T** = α-terpineol, **2P1EA** = 2-phenylethyl acetate, **G** = geraniol, **2PEA** = 2-phenylethanol, **OA** = octanoic acid

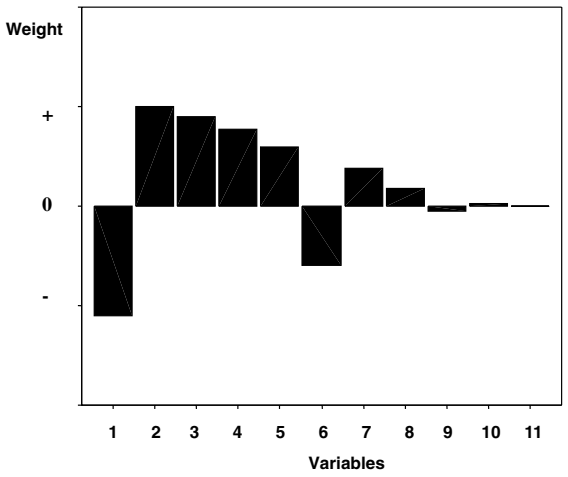

Fig. 2. Results obtained in Plakett-Burman design. (**1** = fibre, **2** = extraction time, **3** = split ratio, **4** = injections per vial, **5** = magnetic stirring, **6** = desorption time, **7** = NaCl, **8** = $(NH_4)_2SO_4$, **9** = K_2SO_4, **10** = Na_2SO_4, **11** = NaH_2PO_4)

esters was good for the PDMS 7 and PDMS 100 fibre, but some alcohols (1-pentanol and 1-hexanol) were not extracted when the PDMS 7 fibre was used. Furthermore, the polyacrylate and CWAX/DVB fibres proved to be sensible choices, although terpenic compounds were not extracted. Therefore, the optimization was performed using PDMS 100 and CWAX/DVB fibres. The bibliography recommended [19] the first fibre, but our results showed that the best extraction was obtained with the second fibre (Fig. 1).

Optimization

Once the preliminary tests were completed, all the parameters affecting the extraction procedure and some parameters affecting the separation were optimized. Some parameters, such as the kind of column (HP-INNOWax) or the temperature program (60 °C for 4 min and then up to 170 °C for 12 min at a rate of 4 °C min^{-1}), were selected on the basis of previous studies [15]. Other chromatographic conditions such as carrier gas flow or flow rates for FID were cited previously. The headspace mode is employed and 0.800 mL of synthetic wine containing the volatile compounds were placed in the vials.

A Plackett-Burman design [26] has been used. This is a special design to estimate only the significant parameters: (n-1) factors can be tested in n runs, where n is a multiple of four. In this work, eleven factors were considered: extraction time, desorption time, split ratio, magnetic stirring, kind of fibre, number of injections per vial, and presence or absence of NaCl, Na_2SO_4, K_2SO_4, $(NH_4)_2SO_4$ and NaH_2PO_4. The salt concentration was 1% (w/v).

As it is known, in a Plackett-Burman design only two levels can be considered for each factor. Table 3 shows the factors and the corresponding levels. These levels were chosen on the basis of the preliminary tests.

Statgraphics Plus 4.0 was used to obtain the experimental matrix (36 runs); two replicates were used and the experiments were randomized. The response signal was the peak area of each compound.

The results obtained in the Plackett-Burman experiment show that the extraction time, the split ratio, the type of fibre, the number of injection per vial and the presence of ammonium sulphate are significative for all volatile compounds. The rest of the factors are significative for almost all volatile compounds with some exception. The weight of each variable is shown in Fig. 2. The weight values for each variable are data given by the soft-

Table 4. Analytical characteristics

Volatile compounds	Slope	Intercept values (95%)	LOD[1] (mg L^{-1})	R.S.D.[2] (%)	Upper Linear Range (mg L^{-1})
3-methyl-1-butyl acetate	0.487 ± 0.023	0.0280 ± 0.0110	0.00300	5.1	300
3-methyl-1-butanol	0.583 ± 0.018	0.0280 ± 0.0160	0.00260	5.7	750
ethyl hexanoate	1.90 ± 0.18	0.418 ± 0.193	0.00800	4.8	220
pentanol	0.406 ± 0.029	0.0530 ± 0.0352	0.00370	1.8	100
hexanol	0.503 ± 0.016	0.0200 ± 0.0121	0.00320	4.1	240
linalool	0.962 ± 0.056	0.126 ± 0.064	0.00160	1.1	110
diethyl succinate	0.603 ± 0.014	0.0190 ± 0.0141	0.00250	4.4	220
α-terpineol	0.802 ± 0.042	0.0670 ± 0.0292	0.00190	5.3	100
2-phenylethyl acetate	0.858 ± 0.045	0.121 ± 0.040	0.00180	5.1	110
geraniol	0.279 ± 0.020	0.0880 ± 0.0232	0.00540	2.4	100
2-phenylethanol	0.978 ± 0.101	0.283 ± 0.025	0.00150	3.5	210
octanoic acid	0.683 ± 0.015	0.0210 ± 0.0131	0.00220	3.1	150

[1]Limit of detection
[2]Relative standard deviation

Table 5. Concentration of volatile compounds found in real wine samples

Volatile compounds	Concentrations (mg L^{-1})				
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
3-methyl-1-butyl acetate	12.5 ± 0.3	10.5 ± 0.3	13.2 ± 0.3	12.7 ± 0.3	10.6 ± 0.3
3-methyl-1-butanol	48.2 ± 0.3	46.2 ± 0.4	39.6 ± 0.4	48.9 ± 0.4	37.2 ± 0.4
ethyl hexanoate	2.70 ± 0.10	2.40 ± 0.10	1.90 ± 0.10	2.30 ± 0.10	2.80 ± 0.10
pentanol	0.0202 ± 0.0001	0.0183 ± 0.0002	0.0104 ± 0.0001	0.0191 ± 0.0006	0.0232 ± 0.0004
hexanol	2.77 ± 0.10	2.65 ± 0.10	3.17 ± 0.10	2.97 ± 0.10	2.87 ± 0.10
linalool	Nd	Nd	Nd	Nd	Nd
diethyl succinate	0.30 ± 0.10	0.281 ± 0.102	0.302 ± 0.101	0.310 ± 0.104	0.292 ± 0.107
α-terpineol	Nd	Nd	Nd	Nd	Nd
2-phenylethyl acetate	3.70 ± 0.10	2.80 ± 0.10	3.60 ± 0.10	3.50 ± 0.10	3.50 ± 0.10
geraniol	Nd	Nd	Nd	Nd	Nd
2-phenylethanol	4.10 ± 0.20	3.90 ± 0.20	4.00 ± 0.20	4.30 ± 0.20	3.70 ± 0.20
octanoic acid	0.300 ± 0.002	0.292 ± 0.003	0.301 ± 0.006	0.201 ± 0.004	0.202 ± 0.004

$n = 3$
Nd: No detected

ware and they are a comparative measure between them. If the value is positive, the best option in the variable is the level + (for example, the best conditions for the extraction time are 10 min, corresponding to level +). As it can be seen, the most significative parameters were the kind of fibre and the extraction time, followed by the split ratio and the number of injections per vial. The other parameters were more or less equally relevant.

The optimum parameters are: extraction time 10 min, desorption time 2 min, split ratio 0.15:1, magnetic stirring, fibre coated with 65 μm of carbowax/divinylbenzene, one injection per vial and two salts, sodium chloride and ammonium sulphate at 1% (*w*/*v*).

Analytical Characteristics

The analytical characteristics were obtained in the optimum conditions. The calibration graphs were constructed with three replicates of six standard solutions (prepared in synthetic wine) within the range of 0.0101-750 mg L^{-1}, using 3-octanol at 15.8 mg L^{-1} as internal standard. These solutions were extracted and analysed by SPME-GC. The regression coefficient, slope and intercept values were calculated by the linear least-squares method. In all cases, a good correlation ($r > 0.99$) was observed. The slope, intercept values, detection limits (calculated as a signal three times the height of the blank measurement background), precision (expressed as the relative standard deviation) and lineal range are shown in Table 4.

Five wine samples from the *Denominación de Origen Calificada* Rioja were analyzed in triplicate following the procedure described. The quantification was made using calibration graphs and the results are shown in Table 5. These calibration graphs were prepared considering the volatile compounds concentration.

Conclusions

Solid-Phase Microextration in the Headspace (HS-SPME) mode and Gas Chromatography with FID detector is a good method for the determination of volatile compounds in wine. It is a simple and rapid procedure, no organic solvents are required and the repeatability of the method is very good. No sample treatment is required.

Acknowledgements

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