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Separation and determination of volatile compounds in synthetic wine samples by gas chromatography using UV-visible molecular absorption spectrometry as detector

T. Cedrón-Fernández [a], C. Sáenz-Barrio [a,*], S. Cabredo-Pinillos [a], I. Sanz-Vicente [b]

[a] *Edificio Científico-Tecnológico, Departamento de Química, (Química Analítica), Universidad de La Rioja, C/Madre de Dios 51, 26006 Logroño, Spain*

[b] *Facultad de Ciencias, Departamento de Química Analítica, Universidad de Zaragoza, Plaza San Francisco s/n, 50009 Zaragoza, Spain*

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Abstract

This paper shows the separation and determination of eight compounds containing the OH group (ethanol, pentanol, 3-methyl-1-butanol, hexanol, phenol, benzyl-alcohol, phenylethanol and geraniol) in synthetic wine by gas chromatography using UV-Vis molecular absorption spectrometry as detection system. All the parameters affecting the separation and determination were optimised using some methods of experiment design. The analytical characteristics of each compound were calculated and detection limits ranging from 2.3 to 74 mg l^{-1} have been obtained.

Keywords: Gas chromatography; EAM-UV-Vis detector; Wine samples; Volatile compounds

1. Introduction

Several volatile compounds have been identified in wine so far [1]. The esters are qualitatively the major constituents of wine, together with water, ethanol and fusel alcohols [2,3].

To determine these constituents, a great deal of methods of isolation and determination have been developed such as headspace sampling [4], liquid–liquid [5], ultrasound [6], supercritical fluid extraction [7] or purge and cold trapping [8] and in recent years, solid phase microextraction [9–11].

A new coupling system based on gas chromatography and UV-Vis molecular absorption spectrometry has been developed by our team [12]. Some theoretical studies have been carried out in order to increase the sensitivity, such as derivatisation [13] or data acquisition and treatment [14]. Benzene derivatives [15], alcohols [16] and polyaromatic hydrocarbons [17] have been analysed by this instrumentation.

* Corresponding author. Tel.: +34-941-299-633; fax: +34-941-299-621.

E-mail address: cecilia.saenz@dq.unirioja.es (C. Sáenz-Barrio).

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The coupling of Gas Chromatography-Gas Phase Molecular Absorption Spectrometry (GC-GPMAS) is seldom reported in the literature. It was mentioned for the first time in 1962 [18] when a heated copper tube was used to transport the samples emerging from the chromatograph to an absorption cell in the spectrophotometer. Later, other studies showing different modifications have been published [19–25] and in 1998 a commercial detector based on a system described by Lagesson et al. [26] appeared.

This study is aimed at illustrating the potential of the GC-GPMAS by determining a group of alcohols present in wine such as ethanol, pentanol, 3-methyl-1-butanol, hexanol, phenol, benzyl-alcohol, phenylethanol and geraniol.

2. Experimental

2.1. Apparatus

A Gas Chromatograph Hewlett-Packard (HP) 5892A equipped with a packed column (4 m × 0.125 inch.) filled with 5% SE-30 on 80/100 Chromosorb W has been applied. The FID was eliminated and the final part of the chromatographic column was removed and connected directly to the flow cell (Hellma 174QS 1 cm) placed in the spectrophotometer.

A diode-array spectrophotometer HP 8451A with some peripherics (HP 98155 keyboard, HP 9121 disk drive for bulk data storage and HP7475A graphics plotter) has been used. In order to avoid the condensation of the volatiles, a HP 89090A Peltier temperature control accessory served for the flow cell heating. A home-made flow cell heater [17] has also been used, and the maximum temperature to be obtained has been 400 °C.

The coupling between the GC and the spectrophotometer is direct, so a home-made temperature controller has been used to heat the part of the column outside the oven in order to maintain the chromatographic resolution.

A Syringe Hamilton model 0084857 was used to inject the compounds in the GC.

The spectrophotometer has been programmed to measure each compound at different wavelengths.

Statgraphics and Unscrambler have also been used.

2.2. Reagents

The solvents used are dichloromethane PA (Merck) and petroleum ether HPLC grade (Lab Scan).

The alcohols and the hydroxylated derivatives are ethanol (Panreac 99.5%), pentanol (Fluka 99%), 3-methyl-1-butanol (Aldrich 98%), hexanol (Fluka $>98\%$), phenol (Carlo Erba 99.5%), benzyl-alcohol (Aldrich 99%), phenylethanol (Fluka $>99\%$) and geraniol (Aldrich 98%). Standard solutions were prepared daily by serial dilution of the stock solution.

2.3. Procedure

The flame ionisation detector has been eliminated from the gas chromatograph. A part of the packed column has been taken outside (about 20 cm more or less) and connected directly to the flow cell placed in the spectrophotometer Peltier accessory (heated at 70 °C). In order to keep the oven and the outside column at the same temperature, a home-made system has been built. Two meters of high temperature heater hook-up wire insulated with silicone rubber have been wound around the external column and connected to a variable transformer. When a temperature program is used for the oven, the setting of the temperature of the external column must be made by hand. A schematic diagram of the GC-UV-Vis spectrophotometer and information about the BASIC programs used are shown in [15]. For all studied alcohols the stock solutions have been made by means of petroleum ether. The chromatographic conditions are 265 °C as injector temperature, 25 ml min^{-1} as nitrogen flow and the column temperature has been held at 90 °C for 1 min and then the speed of heating has been held for 1 min at 20 °C min^{-1} to 170 °C.

2.4. Procedure for the application

The synthetic wine is a solution of ethanol in water at 12% (m V^{-1}), modifying the pH with tartaric acid until a pH between 3 and 4 is obtained. In this particular case, the pH was adjusted at 3.4. To obtain an increase in the sensitivity a microextraction procedure [27] has been used. 1.5 g of ammonium sulphate and 0.6 g of sodium dihydrogen phosphate have been placed in a microextraction tube, 10.0 ml of synthetic wine containing the compounds have been added, and the mixture shaken until the salt was completely dissolved. The solvent, 1.0 ml of petroleum ether, has been added and the resulting mixture shaken in a horizontal rotating agitator for 20 min. Afterwards, the mixture has been centrifuged at 3000 rpm for 10 min. A part of the organic phase has been injected (three times) into the GC using the optimum conditions cited above. This procedure has been repeated three times using different concentration mixtures.

3. Results and discussion

3.1. Solvent choice

Hexane and petroleum ether are good solvents for UV-Vis spectrometry because they do not absorb within the current wavelength range. In this study, petroleum ether has been used because some alcohols were not soluble in hexane. Other solvents showed a high absorbance within the wavelength range used, but could be used if the retention times of the solvent and the compound do not coincide. But if overlapping occurred, it was possible to choose a measurement wavelength where the compound absorbed but not the solvent.

3.2. Molecular absorption spectra

In a previous paper [16] the importance of controlling the flow cell temperature was shown, firstly because the condensation of the compounds must be avoided and secondly because the temperature might influence the molar absorptivity coefficient or cause thermochromic phenomena.

As a first step, the gas phase molecular absorption spectra have been obtained at 70 °C of cell temperature. This parameter was later optimised.

A previous test was necessary to obtain the retention times of the compounds. With this information in hands the gas phase spectra were recorded as shown in Fig. 1. The most important data derived from the spectra is the wavelength of maximum absorbance.

3.3. Parameters optimisation

All the parameters affecting the separation and the determination have been studied. Our group had some experience in this hyphenated technique based on previous tests had already been made. Thus, a commercial flow cell, 1 cm pathlength, has been directly used and petroleum ether as solvent. Nitrogen has been used as carrier gas.

In order to estimate the effect of the experimental parameters on the separation of the compounds, various experimental designs have been used. First of all, a Plackett–Burmann design has been used, which is a special design for estimating only the significant parameters: $(n-1)$ factors can be tested in n runs, where n is a multiple of four.

Seven factors have been initially considered: injection temperature, nitrogen flow, oven temperature program, injection volume, hello (this is a false variable), cell temperature and integration time. This last factor refers to the way of measurement. In the programme used there is a line MEASURE a, b, c, d, where: a = time during which the equipment is measuring, b = total time of each measurement cycle, c = delay time between the execution of the sentence and the start of the measurement, d = total measurement time. (MEASURE 2, 5, 0, 600 means 2 s measuring, 3 s waiting and again 2 s measuring and 3 s waiting, and so on for 600 s. There is not delay time between the execution of the sentence and the start of the measurement).

As it is known, in a Plackett–Burmann design only two levels can be considered for each factor. Table 1 shows the factors and the corresponding levels. These levels have been chosen on the basis of our previous experiences.

Although it was possible to make the experimental matrix by hand, Statgraphics has been applied and the conclusion was that it failed to work in this case (seven factors), since instead of eight, 12 have been obtained. Then, Unscrambler has been used and the experimental matrix obtained is shown in Table 2. One replicate has been used and the experiments were already randomised. If the experimental error has to be analysed by known statistical probability distributions, certain assumptions must be made. These assumptions are: (a) the experimental errors should be independent, i.e. the disturbances leading to the error should occur independently between experimental runs. (b) The experimental error variance should be constant in the experimental domain. However, the chemical system can be influenced by disturbances which are not random and which will produce a systematic error. There is always a risk that the experimental result may be influenced by non-random, time-dependent errors and the remedy is randomisation. This precaution will transform systematic errors into random errors.

In this case, in order to simplify the measure program, the spectrophotometer has been poised

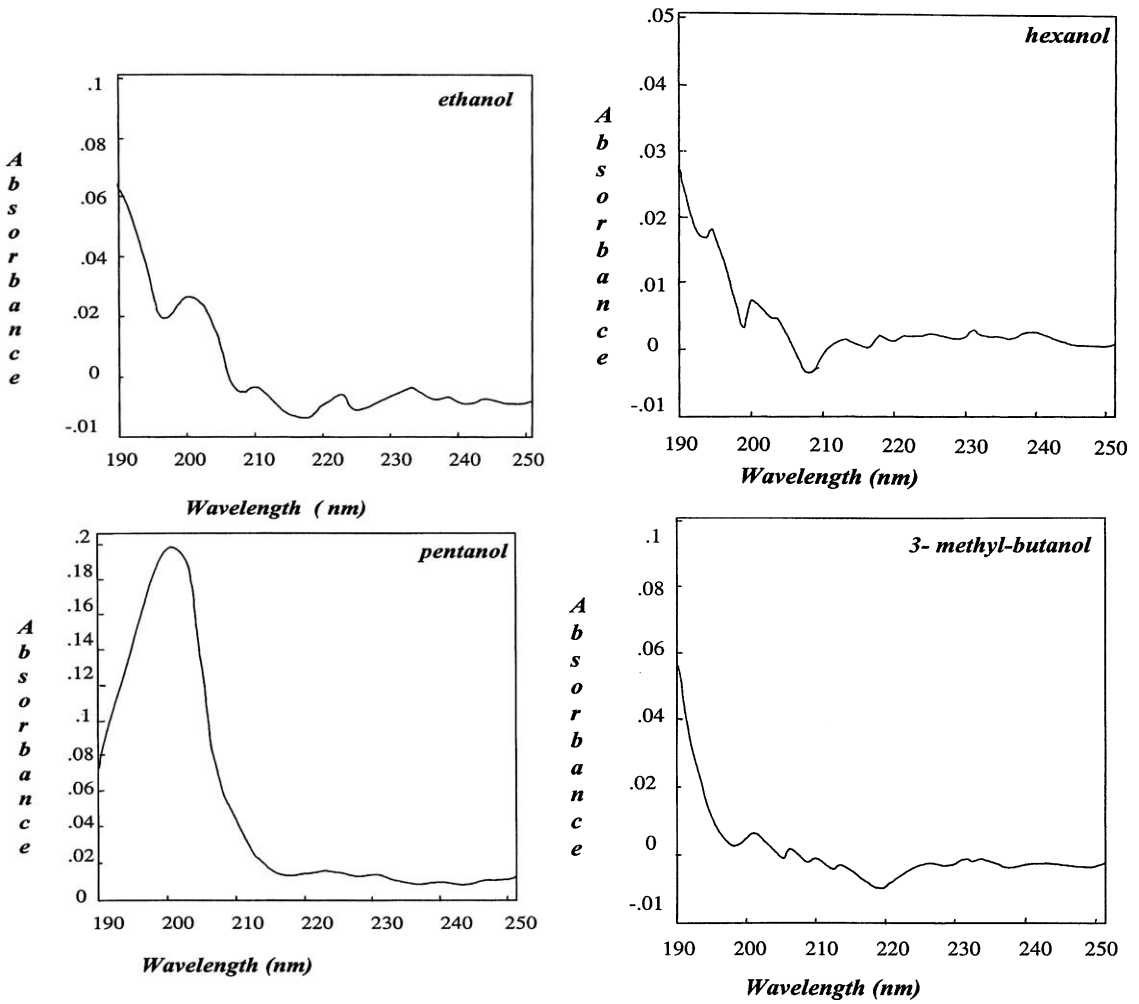

Fig. 1. Gas-phase molecular absorption spectra of ethanol, pentanol, 3-methyl-1-butanol, hexanol, phenol, benzyl alcohol, phenylethanol and geraniol.

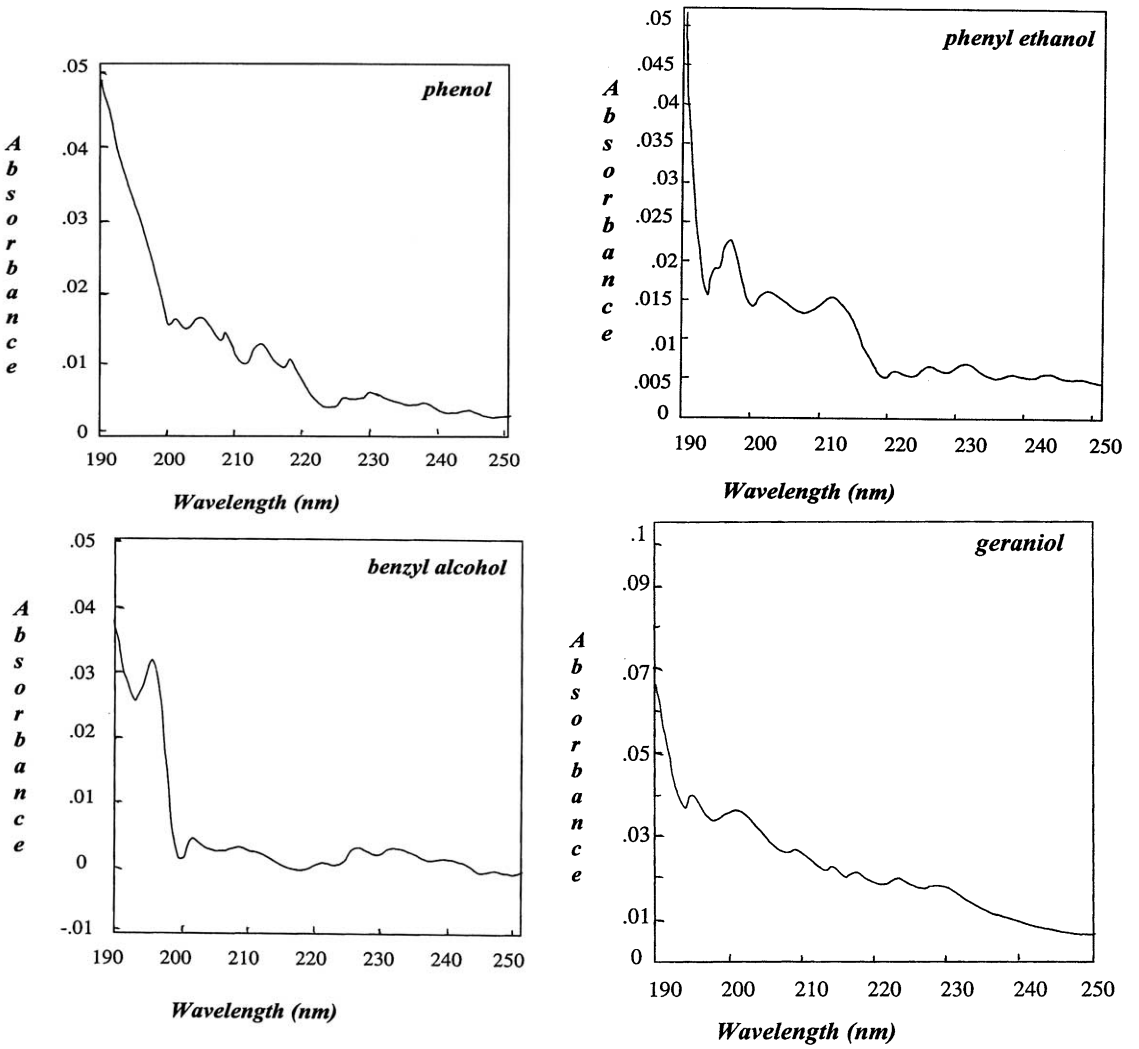

Fig. 1. (*Continued*)

at 190 nm, a wavelength at which all compounds absorb. The signals or the dependent variables are the resolutions between the peaks. There are eight alcohols, so seven resolution values can be obtained: R_{12} (ethanol–pentanol), R_{23} (pentanol–3-methyl-butanol), R_{34} (3-methyl-butanol–hexanol), R_{45} (hexanol–phenol), R_{56} (phenol–benzyl-alcohol), R_{67} (benzyl-alcohol–phenyl-ethyl-alcohol) and R_{78} (phenyl-ethyl-alcohol–geraniol).

The results obtained from the Plackett–Burmann experiment are shown in Table 3. As it can be seen, there are three significant variables: nitrogen flow, oven temperature program and integration time. Two of these factors are continuous (nitrogen flow and integration time) and the third one is discontinuous. Studying the results, no significant differences were observed between the two oven temperature programs used, and so one of them has been chosen (1 min at 90 °C, and afterwards a 20 °C min^{-1} ramp until 170 °C held for 1 min) Then, a new experimental design can only be made with two experimental factors. It has been a full factorial design experiment at two levels with two factors (2^2) plus two central

Table 1
Factors and values considered in the Plackett–Burman design

x_i	Factor	Level (+)	Level (−)
x_1	Injection temperature (°C)	265	300
x_2	Nitrogen flow (ml min^{-1})	25	40
x_3	Oven temperature program (°C)	[a]	[b]
x_4	Injection volume (μl)	30	50
x_5	Hello	Yes	No
x_6	Cell temperature (°C)	70	140
x_7	Integration time (s)	MEASURE 3, 6, 0, 600	MEASURE 2, 5, 0, 600

[a] From 120 °C for 1 min and then at 20 °C min^{-1} to 200 °C (held for 1 min).
[b] From 90 °C for 1 min and then at 20 °C min^{-1} to 170 °C (held for 1 min).

points. One replicate has been made for each experiment, so there are 12 runs. Randomisation has also been made. The inclusion of two central points has allowed to see if a curvature exists. In addition, these two central points together with the replicates helped to estimate the variation coefficients.

This optimisation technique (2^2+2 central points) has the advantage of providing information on the main effects and the interactions between the factors. The dependent variable was the resolution between the chromatographic peaks (obtained at 190 nm), whereas the independent variables were the nitrogen flow (x_1) and the integration time (x_2). The experimental matrix and the model have been obtained using Statgraphics. The response values are shown in Table 4.

The general equation of the model is:

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_1 x_2$$

where y is the predicted response, x_i are the factors and β_i are the adjusted coefficients. In this case, y is the resolution, x_1 is the nitrogen flow and x_2 the integration time. The adjusted coefficient values are shown in Table 5. In all cases, the interaction between the two effects was statistically non-significant, so β_3 was virtually zero. In addition, there were no significant differences between the replicated blocks.

As it can be concluded, the nitrogen flow was the factor, which influenced the resolution to the greatest extent, and appeared with negative sign. The integration time was, for all the responses, statistically significant and positive, and the interaction between the two effects was statistically non-significant.

Taking this study and the previous experiments into account, the conditions considered as optimum are shown in Table 6. Under these conditions, the chromatogram obtained is shown in Fig. 2.

Table 2
Experimental matrix with the Plackett–Burman design

Runs	Factors						
	x_1	x_2	x_3	x_4	x_5	x_6	x_7
1	+	+	+	+	+	+	−
2	+	−	+	+	−	−	+
3	−	+	−	+	+	−	+
4	+	−	−	−	+	−	−
5	−	+	+	−	−	−	−
6	+	−	−	−	+	−	−
7	+	−	+	+	−	−	+
8	−	−	+	−	+	+	+
9	+	+	+	+	+	+	−
10	+	+	−	−	−	+	+
11	−	+	−	+	+	−	+
12	+	+	−	−	−	+	+
13	−	+	+	−	−	−	−
14	−	−	+	−	+	+	+
15	−	−	−	+	−	+	−
16	−	−	−	+	−	+	−

x_1 = injection temperature, x_2 = nitrogen flow, x_3 = oven temperature, x_4 = injection volume, x_5 = hello, x_6 = cell temperature, x_7 = integration time.

Table 3
Results obtained in the Plackett–Burmann experiment

Factor	R_{12}	R_{23}	R_{34}	R_{45}	R_{56}	R_{67}	R_{78}
Injection temperature (°C)	NS	NS	NS	NS	NS	NS	NS
Nitrogen flow (ml min^{-1})	S	S	S	S	S	S	S
Oven temperature program (°C)	S	S	S	S	S	S	S
Injection volume (μl)	NS	NS	NS	NS	NS	NS	NS
Hello	NS	NS	NS	NS	NS	NS	NS
Cell temperature (°C)	NS	NS	NS	NS	NS	NS	NS
Integration time (s)	S	S	S	S	S	S	S

S, significative; NS, no significative.

Table 4
Results obtained in the 2^2+2 central points experiment

Runs	Normalised points		Variables		Resolution						
	x_1	y_1	Flow	Int. time	R_{12}	R_{23}	R_{34}	R_{45}	R_{56}	R_{67}	R_{78}
1	−	−	25	2, 5, 0, 600	4.2	1.6	4.4	5.0	1.6	3.1	3.1
2	0	0	36	3, 7, 0, 600	3.7	1.0	4.3	5.0	1.7	2.3	4.3
3	0	0	36	3, 7, 0, 600	3.6	1.1	3.9	4.5	1.6	2.2	3.9
4	+	+	40	3, 6, 0, 600	3.3	1.4	4.0	3.7	2.0	2.0	2.2
5	+	−	40	2, 5, 0, 600	2.2	1.0	2.5	3.1	1.7	1.4	2.3
6	−	+	25	3, 6, 0, 600	4.8	1.7	4.7	5.1	3.0	1.7	3.5
7	−	−	25	2, 5, 0, 600	4.2	1.5	4.1	4.9	1.7	2.9	3.1
8	0	0	36	3, 7, 0, 600	3.6	1.1	4.0	4.6	2.0	2.2	4.0
9	0	0	36	3, 7, 0, 600	3.7	1.1	4.1	4.9	1.7	2.2	4.2
10	+	+	40	3, 6, 0, 600	3.3	1.4	4.1	3.7	2.1	2.1	2.3
11	+	−	40	2, 5, 0, 600	2.2	1.0	2.6	3.2	1.8	1.5	2.4
12	−	+	25	3, 6, 0, 600	4.9	1.5	4.6	4.9	2.8	1.9	3.4

3.4. Analytical characteristics

Under the instrumental and chemical optimal conditions obtained in the experimental section, calibration studies have been made for each compound.

The solutions containing the eight alcohols have been injected and the absorbance values for each peak have been considered as quantification parameter. For each compound, five solutions of different concentration have been prepared in petroleum ether by serial dilution of the stock solutions. Five mixtures prepared using the solutions above has been used. In eight boxes (one for each alcohol) we have placed five pieces of paper, one for each concentration value. Then, for mixture 1, a piece of paper from each box has been drawn at random, and we have proceeded in the same way for the rest of the mixtures.

Table 7 shows the slopes of the calibration curves (sensitivity), the detection limits (calculated as a signal three times as much as the height of

Table 5
Coefficient values in the 2^2+2 central points experiment

	β_0	β_1	β_2
R_{12}	3.15	−0.108	0.0153
R_{23}	1.45	−0.0268	0.00272
R_{34}	1.70	−0.0676	0.0166
R_{45}	5.13	−0.0877	0.00820
R_{56}	0.178	−0.0276	0.0113
R_{67}	4.37	−0.0353	0.00380
R_{78}	2.62	−0.0390	0.00705

Table 6
Optimum conditions

Parameters	
Injection temperature (°C)	265
Nitrogen flow (ml min^{-1})	25
Oven temperature program (°C)	[a]
Injection volume (μl)	50
Cell temperature (°C)	70
Integration time (s)	MEASURE 3, 6, 0, 600

[a] From 90 °C for 1 min and then at 20 °C min^{-1} to 170 °C (held for 1 min).

Table 7
Analytical characteristics

Compound	Sensitivity ($l\ mg^{-1}$)	DL ($mg\ l^{-1}$)	R.S.D. (%)
Ethanol	1.08×10^{-4}	62	3
1-Pentanol	1.02×10^{-4}	66	8
3-Methyl-1-Butanol	9.03×10^{-5}	74	6
1-Hexanol	9.51×10^{-5}	71	4
Phenol	2.85×10^{-3}	2.3	2
Benzyl-alcohol	5.95×10^{-4}	11	5
Phenylethanol	8.31×10^{-4}	8.1	4
Geraniol	4.21×10^{-4}	16	6

DL = Detection Limit, R.S.D. = Relative Standard Deviation.

the background of the blank measurement), and the relative standard deviation values.

3.5. Application

A synthetic wine has been used as matrix and the eight alcohols have been spiked in it. In all the cases the extraction yields were between 30 and 48%, except for 3-methyl-1-butanol (17%). Benzyl-alcohol was extracted with yields higher than 100%. We have tried to obtain the extraction yields following the same procedure but injecting them into a conventional gas chromatograph with FID. The results were not good because petroleum ether was not an adequate solvent.

Three white Rioja wines were spiked with the eight compounds and analysed using the same procedure. In all the cases, the extraction yields were similar except for hexanol, which goes from 48% for the synthetic wine to 68% in the case of real wine.

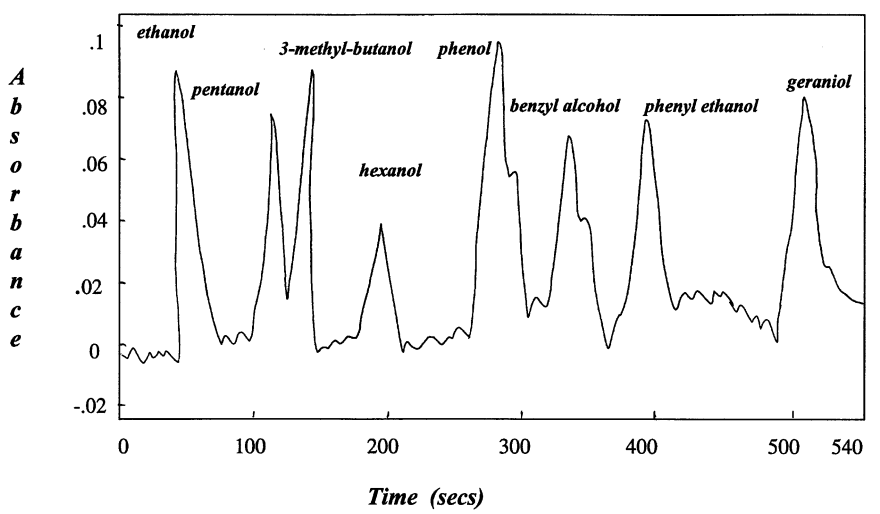

Fig. 2. Chromatogram obtained using the optimum conditions.

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