

Short communication

# Determination of important odor-active aldehydes of wine through gas chromatography–mass spectrometry of their *O*-(2,3,4,5,6-pentafluorobenzyl)oximes formed directly in the solid phase extraction cartridge used for selective isolation

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## Abstract

A method for the quantitative determination of octanal, nonanal, decanal, (*E*)-2-nonenal and (*E*, *Z*)-2,6-nonadienal in wine has been developed. In the proposed method, 200 ml of wine percolate through a solid phase extraction (SPE) cartridge packed with 200 mg of LiChrolut EN resins. The interferences are eluted with 60 ml of an aqueous solution containing 40% of methanol and 1% of NaHCO<sub>3</sub>. In the same SPE cartridge, the corresponding *O*-(2,3,4,5,6-pentafluorobenzyl)oximes are formed by letting percolate 2 ml of the reagent solution (5 mg ml<sup>-1</sup>). At room temperature the derivatization goes to completion in 15 min. The derivatives are eluted with 2 ml of dichloromethane, and the extract is concentrated and then analyzed by gas chromatography–mass spectrometry (GC–MS). The percentage of recovery in the isolation process is better than 90% in all cases with the exception of octanal, and is independent of the wine studied. In the cases of octanal, nonanal and decanal, the detection limits of the method are determined by the contamination levels of the reagent itself, and vary between 160 and 380 ng l<sup>-1</sup>. For (*E*)-2-nonenal and (*E*, *Z*)-2,6-nonadienal, the detection limits were 12 and 20 and ng l<sup>-1</sup>, respectively. The linearity of the method upheld until 10 µg l<sup>-1</sup> and was satisfactory in all cases. The reproducibility of the method is independent of the concentration and ranges from 30 to 190 ng l<sup>-1</sup>. The method has been applied to the analysis of these components in several wine samples. With the exception of (*E*, *Z*)-2,6-nonadienal, all the components can reach concentrations above their corresponding odor threshold values.

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## 1. Introduction

Aldehydes are volatile compounds widely distributed in foods and beverages. Most of them have significant sensory properties and can impart pleasant or unpleasant notes to different products [1]. A study of their sensory properties reveals that those with 8–10 carbon atoms, such as (*E*)-2-nonenal, octanal, nonanal, decanal or (*E*, *Z*)-2,6-nonadienal, are strong odorants [2–6]. The odor properties of (*E*)-2-nonenal are particularly important, since it can be responsible for important off-flavors [7,8]. In the case of wine, (*E*)-2-nonenal seems to be the cause of a “sawdust” or “plank” off-flavor [9,10], while the exact role of the other aldehydes in the aroma of wine is not clearly

understood. Since this is mainly due to the scarcity of available data, it seems sensible to develop methods to quantify these compounds.

Due to the poor chromatographic and MS properties of higher aldehydes, most analytical methods are based on gas chromatography–mass spectrometry (GC–MS) or GC–ECD analysis of chemical derivatives. In the case of wine, only for (*E*)-2-nonenal there are some reports on the analysis of the underivatized molecule [11]. The chemical derivatization of higher aldehydes in wines has to face the fact that wine contains an extremely high amount of light carbonyls. The major aldehyde of wine is acetaldehyde whose concentration can be as high as 300 mg l<sup>-1</sup> [12]. Some wines can also contain high levels of some other carbonyls, such as pyruvic acid (up to 460 mg l<sup>-1</sup>), hydroxy-butanone-acetoin (up to 200 mg l<sup>-1</sup>), 2,3-butanedione-diacetyl (up to 5 mg l<sup>-1</sup>) and C3–C5 aliphatic aldehydes (up to 5 mg l<sup>-1</sup>) [12–14]. The

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expected levels of (*E*)-2-nonenal and other higher aldehydes are, however, between 0.1 and 5  $\mu\text{g l}^{-1}$  [9,14].

Most of the methods previously developed for the analysis of carbonyl components in wine are based on the GC–MS or GC–ECD analysis of oximes formed by reaction with *O*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride (PFBHA), although other derivatization reagents have been explored, such as 2,4-dinitrophenyl-hydrazine [9] or cysteamine [15,16]. The direct derivatization of carbonyls in wine has been proposed as a method to determine some light carbonyls [17]. On the other hand, the analysis of (*E*)-2-nonenal in wine, or of some higher methyl ketones in cognac, has been carried out by performing the chemical reaction over an organic extract obtained from the wine or cognac [10,18]. In a more recent proposal, neutralized wine is initially percolated through an anionic exchange column in order to eliminate the interference cause by pyruvic acid [14]. However, none of these strategies results in good separation between the higher carbonyls and the major lighter carbonyls. In addition, all those methods use tedious liquid–liquid extractions.

On the other hand, several authors have shown the potential of direct derivatization of carbonyls on a solid phase, particularly in SPME-based strategies [19–21], or in air analysis [22]. However, the sensitivity achieved with the more often used head space SPME is not satisfactory for aldehydes with more than six carbon atoms [23], unless the sample can be heated to high temperatures, as is the case of edible oils [24]. In the present work, we have explored the possibility of preparing the derivatives directly on a solid phase extraction (SPE) bed in which the targeted analytes have been previously isolated and pre-concentrated. The result is a method that allows to quantify higher aldehydes of wine; we also think that this strategy can be of interest in the analysis of similar compounds in different matrixes.

## 2. Material and methods

### 2.1. Reagents, samples and standards

Octanal 99%, decanal 95%, (*E*)-2-nonenal 97% and (*E*, *Z*)-2,6-nonadienal 95% were from Aldrich–España (Madrid, Spain), nonanal 97.5% was supplied by Polyscience (Niles, IL, USA). 2-Octanol and 2-undecanone (>97%), used as internal standard and surrogate standard respectively, were purchased from Fluka–España (Madrid, Spain). *O*-(2,3,4,5,6-Pentafluorobenzyl)hydroxylamine hydrochloride ( $\geq 99\%$ ), used as a derivatization reagent, was purchased also from Fluka. The 5  $\text{mg ml}^{-1}$  aqueous solution of PFBHA was prepared daily.

Dichloromethane HPLC-quality was from Fisher Chemicals (Leicester, UK), methanol HPLC-grade was from Merck (Darmstadt, Germany), ethanol absolute, potassium hydrogen phthalate and sodium hydrogen carbonate, all Analytical Reagent Grade, were from Panreac (Barcelona, Spain),

sulfuric acid (95–97%, synthesis grade) was from Scharlau (Barcelona, Spain). Pure water was obtained from a Milli-Q purification system (Millipore, Bedford, USA). LiChrolut EN resins (styrene-vinylbenzene, divinylbenzene polymer), prepaced in 200 mg cartridge (3 ml total volume) were obtained from Merck (Darmstadt, Germany). Three wine samples (two young red wines and a young white wine) were used in the development and validation of the method. The method was further applied to different wines made with Merlot, Chardonnay or a mixture of grape varieties.

Semiautomated solid phase extraction was carried out with a VAC ELUT 20 station from Varian (Walnut Creek, USA).

### 2.2. Gas chromatography–mass spectrometry

GC–MS was carried out on a Varian CP-3800 Saturn 2200 gas chromatograph ion trap–mass spectrometric detection system. The column was a DB-WAX (crosslinked Carbowax 20M) from J & W Scientific (Folsom, USA), 60 m  $\times$  0.25 mm i.d., with 0.25  $\mu\text{m}$  film thickness, and was preceded by a 3 m  $\times$  0.25 mm uncoated precolumn (deactivated to give an intermediate polarity surface). The carrier gas was He at a constant flow of 1  $\text{ml min}^{-1}$  (average linear velocity of 25  $\text{cm s}^{-1}$ ). One microlitre of extract was injected in splitless mode, with a pulse pressure of 40 psi for 1.50 min. The splitless time was also 1.5 min. The chromatographic oven was held at 40  $^{\circ}\text{C}$  for 5 min, then raised to 140  $^{\circ}\text{C}$  at 10  $^{\circ}\text{C min}^{-1}$ , then to 190  $^{\circ}\text{C}$  at 2  $^{\circ}\text{C min}^{-1}$  and finally to 210  $^{\circ}\text{C}$  at 20  $^{\circ}\text{C min}^{-1}$ . A 45–350  $m/z$  mass range was recorded in full-scan mode, except in the cases of (*E*)-2-nonenal and (*E*, *Z*)-2,6-nonadienal. In these cases, the selected ion storage (SIS) feature of the ion trap was used to gain sensitivity. The 176–255 range of ions was recorded in these two cases.

### 2.3. Proposed method

Two hundred milliliter of wine, previously spiked with 0.01 ml of 2-undecanone 10  $\text{mg l}^{-1}$  standard solution in ethanol (surrogate), are loaded onto a 200 mg LiChrolut-EN solid phase extraction cartridge (previously conditioned with 4 ml dichloromethane, 4 ml methanol and 4 ml of a 13% ethanol (v/v) aqueous solution). Low molecular weight carbonyls, together with the majority of wine volatiles are removed by cleanup with 60 ml of a 40% methanol (v/v) aqueous solution containing 1%  $\text{NaHCO}_3$ . Carbonyls retained in the cartridge are directly derivatized by passing through 2 ml of an aqueous solution of PFBHA (5  $\text{mg ml}^{-1}$ ), and letting the cartridge imbibed with the reagent 15 min at room temperature. Excess of reagent is removed with 10 ml of a 0.05 M sulfuric acid solution. Analytes are finally eluted with 2 ml of dichloromethane. Thirty microliter of internal standard solution (2-octanol 60  $\text{mg l}^{-1}$  in dichloromethane) were added to the extract, which is then concentrated to 100  $\mu\text{l}$  by evaporation in a centrifuge tube heated to 47  $^{\circ}\text{C}$ .

A blank must be prepared daily by applying the whole procedure to a cartridge. The ionic peak areas (sum of the areas from the two isomer peaks) corresponding to the  $m/z$  fragments indicated in Table 3 are first normalized to those of 2-octanol, and then further corrected by subtracting the relative peak areas measured in the blank. These relative peak areas are interpolated in the calibration graphs built as follows. A known mass of analyte is dissolved in a 10 ml aqueous solution containing 10% methanol. This volume is then loaded onto a 200 mg LiChrolut EN cartridge. The oximes are formed and eluted as in the standard procedure. The result for octanal must be corrected by the corresponding recovery given in Table 4, since this compound is not totally retained in the SPE bed. The peaks of 2-undecanone are used as quality control. This compound was selected because of its similarity in behavior (extractability and reactivity with PFBHA) with the analytes. Under these conditions, the ratio of the sum of the areas of the two isomer peaks for the 2-undecanone oxime relative to those of 2-octanol should be  $200 \pm 15$ .

#### 2.4. Method development and validation

##### 2.4.1. Preliminary experiments

We have studied the applicability of some of the methods proposed in the bibliography for the determination of the targeted aldehydes [10,17]. The liquid–solid analyte–resin distribution coefficients and their use in estimating the breakthrough volumes were determined by following the procedure described in references [25,26]. The recovery on the SPE bed and the optimization of the solution and washing volume were performed by analyzing 200 ml of a wine spiked with  $30 \mu\text{g l}^{-1}$  of analyte.

Derivatization: In preliminary experiments, method EPA 556 [27] was applied to the analysis of aqueous solutions containing different percentages of methanol and  $15 \mu\text{g l}^{-1}$  of aldehydes. Combinations of different solvents (water, methanol, pentane, ether, dichloromethane) to elute the analytes isolated on the SPE bed and perform the derivatization reaction were investigated. In order to optimize the derivatization on the bed, wine spiked with  $20 \mu\text{g l}^{-1}$  of the analytes was used. Several concentrations of reagent (2, 5 and  $15 \text{ mg ml}^{-1}$ ) and reaction times (5, 15, 30, 60, 120, 180 and 720 min) were studied. A range of solvents was tried (methanol, dichloromethane, pentane and mixtures of these last two) to elute the oximes formed.

##### 2.4.2. Blanks

In order to improve the blank signals, purification studies of the different reagents were conducted (previous oxime or aldehyde extraction with pentane or dichloromethane, water purification by percolation on a SPE bed, distillation of solvents, prior cleaning with activated charcoal . . .), and different qualities and brands of solvents and of the derivatization reagent were tried. Under the final conditions, a study of repeatability and reproducibility of the

blank signals was conducted during four different working days.

##### 2.4.3. Method validation

Precision was evaluated by means of a triplicate analysis of six different wines. Linearity was studied by standard addition as well as by the derivatization of known amounts of analytes placed in the cartridge. This was done by percolating 10 ml of an aqueous solution (10% methanol) containing known amounts of analytes. In order to evaluate the existence of matrix effects, and to determine the degree of recovery of the method, an experiment of standard recovery was carried out on three different wines.

### 3. Results and discussion

#### 3.1. Isolation and pre-concentration

Direct derivatization in the wine did not result in sufficiently sensitive and selective signals, even after introducing a cleanup step, as it is shown in Fig. 1. Strong interferences made it difficult to obtain unambiguous signals for octanal and (*E*)-2-nonenal. Under these conditions, detection limits for these compounds were estimated as 1.5 and  $0.5 \mu\text{g l}^{-1}$  respectively. In the cases of nonanal and decanal, the method was more selective, but imprecision was still high (R.S.D. between 25 and 35% for  $6 \mu\text{g l}^{-1}$ ). It seems therefore sensible to introduce a pre-separation step before derivatization.

We chose LiChrolut EN resins for this purpose, due to their exceptional capacity to extract slightly polar compounds from wine [26,28]. Table 1 shows the liquid–solid distribution coefficients of analytes and potential interferences between wine and those resins. It can be seen that analytes, are better retained than interferences. The Lövkist–Johnsson model [29] applied to such data [25,26,30] predicts breakthrough volumes higher than 300 ml (for a 200 mg bed), and therefore, a 200 ml loading volume was selected. A recovery experiment confirmed the estimations from the model, as is also shown in Table 1.

The potential interference caused by lighter carbonyls and some other major wine volatiles can be eliminated from the cartridge by washing up with a methanol/water (40% (v/v)) solution containing 1% of  $\text{NaHCO}_3$ . A 60 ml volume of this washing up solution still results in high recovery of analytes (only in the case of octanal is recovery below 90%) while completely eliminating such interferences.

#### 3.2. Derivatization

At the beginning of the study, it was decided to use derivatization conditions as close as possible to those used in method EPA 556 [27], since its protocol of derivatization is one of the best studied procedures. However, such protocol cannot be directly applied to carbonyls retained on a SPE cartridge, and the necessary modifications for

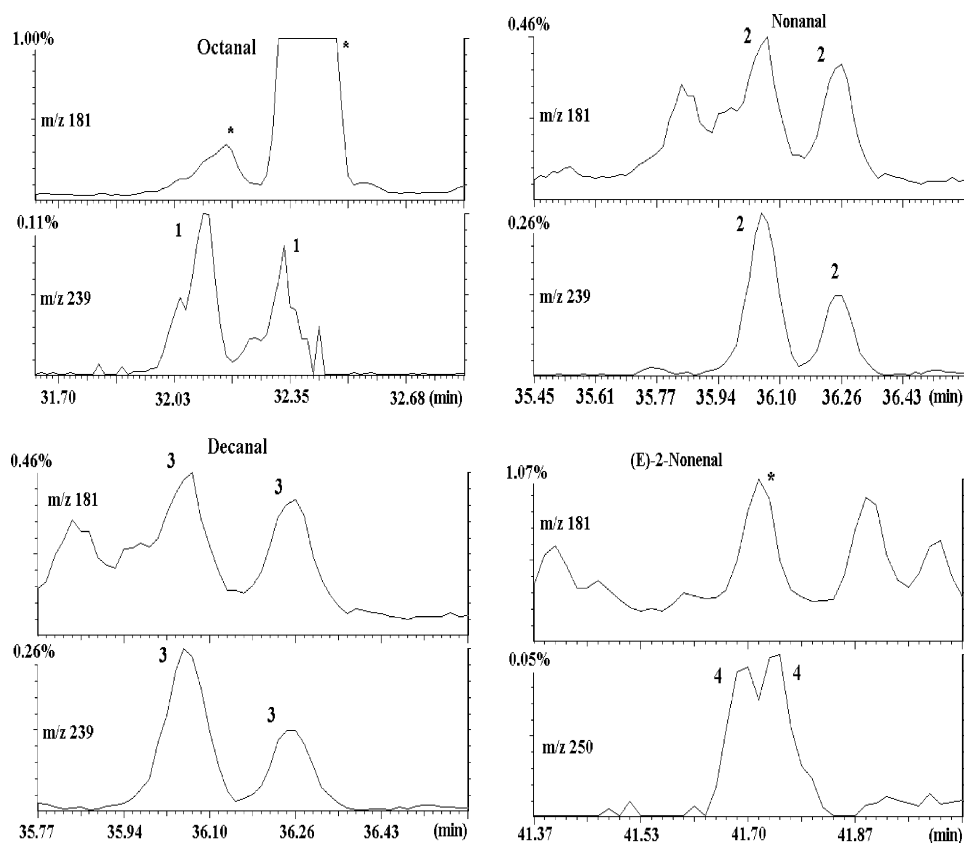


Fig. 1. Chromatogram showing the strong interferences found when derivatization is carried out directly in the wine. The sample (100 ml) was a young red wine spiked with  $6 \mu\text{g l}^{-1}$  of each analyte. After direct derivatization, oximes were retained in a 200 mg LiChrolut EN cartridge, washed up with 60 ml of a 40% methanol/water solution containing 1% of  $\text{NaHCO}_3$  and analytes were finally eluted with 2 ml of dichloromethane. Peaks: 1, octanal-PFBHA; 2, nonanal-PFBHA; 3, decanal-PFBHA; 4, (*E*)-2-nonenal-PFBHA. Peaks marked with an asterisk are interferences. Double peaks occur because two isomers are obtained from each analyte.

its adaptation resulted in a method extremely tedious and hardly reproducible (R.S.D. (%) in all cases above 11%); it was therefore decided to study the progress of the reaction on the SPE bed itself. The reaction on the SPE bed takes place by simply letting 2 ml of the reagent solution percolate by gravity and leaving the cartridge imbibed in this solution for some time. The reaction is almost instantaneous at room temperature; after 5 min, 90% of the reaction has taken place, and after 15 min the reaction is complete (data not shown). No variation in the signal is observed with reaction times of up to one hour. Additionally, longer times seem to be associated with the decomposition of the oximes retained on the SPE bed. The amount of oxime recovered from the cartridge after 2 or after 12 h of reaction was respectively only 60% or only 30% of the amount obtained after 15 min.

As for the concentration of reagent, three different concentrations (2, 5 and  $15 \text{ mg ml}^{-1}$ ) were tested. Results showed that  $5 \text{ mg ml}^{-1}$  represents a fair optimum, since lower concentrations do not guarantee a complete reaction, and higher values produce much higher noise. Regarding the elution solvent, dichloromethane was found to be the most suitable.

The oximes formed in the cartridge were quantitatively recovered in only 2 ml of this solvent, and the extract obtained was much cleaner than when using methanol. On the contrary, pentane hardly showed any capacity to elute the oximes retained on the SPE bed.

### 3.3. Spectrometric signal

The spectrometric signal for the oximes of the five aldehydes studied, obtained in the GC–ion trap–MS analysis of the extracts produced according to the proposed protocol, does not constitute a limiting factor in terms of both sensitivity and selectivity. The absolute limit of detection of the method, determined as the minimum theoretical concentration of an aldehyde detectable in wine, stands within the  $10\text{--}20 \text{ ng l}^{-1}$  range in full-scan mode, which can be considered sufficient. The use of some techniques of ion preparation available in the ion trap should allow the reduction of this limit. Nevertheless, the fact that the limit of real detection comes restricted by the presence of impurities in the blank leads to no effective advantage by the use of these functions.

Table 1  
Retention properties of carbonyls and some major wine volatiles in LiChrolut EN resins

	$K^a$	$V_b$ 10% <sup>b</sup> (ml estimated)	$R$ (%) <sup>c</sup>	$R$ (%) <sup>d</sup>
Acetaldehyde	<15	1	3	0
Acetoine	<15	1	5	0
Ethyl pyruvate	27	2	7	0
Diacetyl	42	4	15	0
Isoamyl alcohol	88	8	31	0
$\beta$ -Phenylethanol	441	38	56	0
Hexanoic acid	954	83	75	0
Butanal	35	4	14	0
Hexanal	607	51	53	8
Octanal	4357	377	98	89
Nonanal	9500	823	98	98
Decanal	10932	947	101	100
( <i>E</i> )-2-Nonenal	8704	754	100	99
( <i>E, Z</i> )-2,6-Nonadienal	8230	713	99	96

Solid–liquid distribution coefficients, predicted breakthrough volumes, and measured recoveries.

<sup>a</sup>  $K$  is the solid–liquid distribution coefficient of the analyte between wine and the sorbent. Its calculation and use is shown in the references [25,26,30].

<sup>b</sup>  $V_b$  10% is the estimated breakthrough volume and corresponds to the volume of wine loaded into the cartridge for which the mass of unretained analyte is 10% [25,26,30].

<sup>c</sup> Recovery in the extraction of 200 ml of wine (spiked with  $30 \mu\text{g l}^{-1}$  of aldehydes) loaded in a 200 mg-SPE cartridge. Elution was carried out with 2 ml of dichloromethane.

<sup>d</sup> As in c, but including a washing up with 60 ml of a methanol/water (40% (v/v)) solution containing 1%  $\text{NaHCO}_3$ .

### 3.4. Validation of the method

#### 3.4.1. Blanks and limits of detection

Obtaining a good blank has been one of the most important problems in the validation of the proposed method. Method EPA 556 [27] already places particular emphasis on this question, although acetaldehyde and formaldehyde are mentioned as the major interferences in water. In our case the problem is caused by the omnipresence of the oximes of octanal, nonanal and decanal in all the blanks studied. An extensive study was conducted to find the origin of the contamination, by studying the solvents, cleaning procedures, water, the derivatization reagent and the rest of the reagents used in the procedure. Our initial hypothesis was that most of this contamination is present in the reagent itself, since the level of contamination seemed to diminish when the concentration of derivatizing agent was reduced. Nevertheless, none of the purification operations attempted either on the derivatization reagent or on the rest of the reagents resulted in any improvement. In addition, the absolute value of the blank oscillates remarkably from day to day, a fact that suggests that unknown environmental factors may affect the blank. The levels of analyte measured in the blanks, given as aldehyde in the sample, were  $0.63 \pm 0.10 \mu\text{g l}^{-1}$  octanal,  $2.47 \pm 0.18 \mu\text{g l}^{-1}$  nonanal,  $1.20 \pm 0.13 \mu\text{g l}^{-1}$  decanal and  $0.031 \pm 0.008 \mu\text{g l}^{-1}$  (*E*)-2-nonenal. Practical implications of these observations are: (a) the need to include

a blank per batch of analyzed samples; (b) limits of detection are defined by the standard deviation of blanks. These data are presented in Table 2.

#### 3.4.2. Figures of merit

Table 2 also shows the global precision of the proposed procedure. The deviations seem to be independent of the analyte concentration, and ranged from 30 to  $190 \text{ ng l}^{-1}$ , which can be considered sufficiently small for the purposes of the method. (*E, Z*)-2,6-nonadienal was not found in any of the wines studied, therefore its precision was determined by replicated analysis of a wine spiked with  $0.2 \mu\text{g l}^{-1}$ . The linearity of the method was determined by the analysis of fortified samples and is shown in Table 3. The linearity was excellent in all the cases and was maintained from the limit of detection up to near  $10 \mu\text{g l}^{-1}$ , in the case of octanal, nonanal and decanal, and up to 3 and  $5 \mu\text{g l}^{-1}$  in the cases

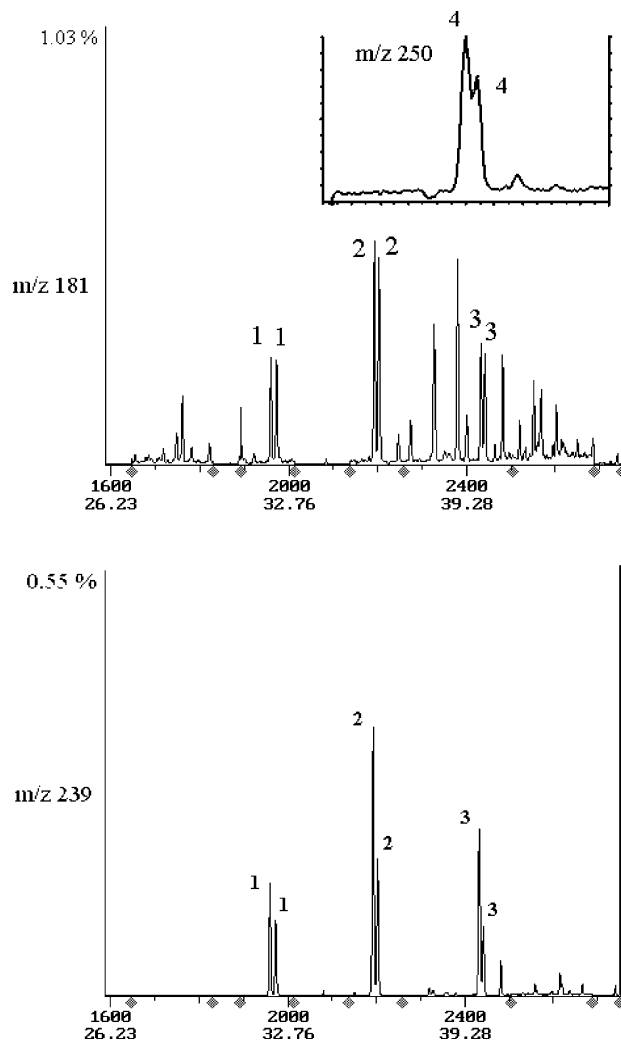


Fig. 2. GC–MS chromatograms of a Merlot wine showing the detail of the different peaks. Peaks: 1, Octanal-PFBHA; 2, Nonanal-PFBHA; 3, Decanal-PFBHA; 4, *E*-2-Nonenal-PFBHA. The concentration of these compounds was ( $\mu\text{g/l}$ ): octanal, 0.972; nonanal, 3.301; decanal, 1.003; (*E*)-2-nonenal, 0.036. Double peaks occur because two isomers are obtained from each analyte.

Table 2  
Method validation 1

	Analytical characteristics of the blanks				Method precision	
	Average signal <sup>a</sup> ( $\mu\text{g l}^{-1}$ )	Intra-day blank reproducibility (S.D.) ( $\mu\text{g l}^{-1}$ )	Inter-day blank reproducibility (S.D.) ( $\mu\text{g l}^{-1}$ )	LD ( $\mu\text{g l}^{-1}$ )	Total S.D. <sup>b</sup> ( $\mu\text{g l}^{-1}$ )	Range of concentration <sup>c</sup> ( $\mu\text{g l}^{-1}$ )
Octanal	0.63	0.08	0.20	0.16 <sup>d</sup>	0.03	<0.2–0.97
Nonanal	2.47	0.19	0.92	0.38 <sup>d</sup>	0.19	1.48–3.30
Decanal	1.20	0.13	0.25	0.26 <sup>d</sup>	0.12	0.65–1.00
( <i>E</i> )-2-Nonenal	0.031	0.006	0.02	0.012 <sup>d</sup>	0.088	<0.016–0.40
( <i>E, Z</i> )-2,6-Nonadienal	–	–	–	0.020	0.063 <sup>e</sup>	<0.024

Analytical characteristics of the blank and method precision.

<sup>a</sup> Average signal calculated with 12 replicates.

<sup>b</sup> Standard deviation obtained by analyzing three replicates of six wines samples.

<sup>c</sup> The range of concentrations found in the wines.

<sup>d</sup> Detection limit estimated as the amount of analyte in a wine that causes a signal twice above the average standard deviation of the blank.

<sup>e</sup> Determined in wine spiked with  $0.2 \mu\text{g l}^{-1}$  ( $n = 5$ ).

Table 3  
Method linearity data and selective *m/z* fragments used for quantitative analysis

	<i>m/z</i>	Slope	$R^2$	Calibrated range ( $\mu\text{g l}^{-1}$ )
Octanal	239	44.85	0.9934	0.2–10
Nonanal	181	52.56	0.9944	0.5–10
	239	27.87	0.9906	
	181 + 239	80.30	0.9931	
Decanal	181	82.76	0.9882	0.3–9
	239	48.15	0.9832	
	181 + 239	130.6	0.9857	
( <i>E</i> )-2-Nonenal	181	81.04	0.9929	0.03–3
	250	105.4	0.9984	
( <i>E, Z</i> )-2,6-Nonadienal	250	9.97	0.9972	0.05–5

of (*E*)-2-nonenal and (*E, Z*)-2,6-nonadienal, respectively. In order to check for the existence of matrix effects a recovery study was conducted. Known amounts of analyte were added to three different wines, and the measured signal increments were compared with the signal obtained after derivatization of equivalent amounts of the analyte on a SPE bed. Results, shown in Table 4, indicate that only in the case of octanal recovery is incomplete, although it is constant and independent of the wine.

### 3.5. Analysis of wines

The method proposed here has been applied to the determination of these components in different wines. The re-

sults are displayed in Table 5; Fig. 2 shows a chromatogram detailing the quantification of the different components. (*E, Z*)-2,6-nonadienal was not found in any of the wines analyzed. The levels of octanal varied between the detection limit and  $0.97 \mu\text{g l}^{-1}$ , its perception threshold being  $0.7 \mu\text{g l}^{-1}$  [31]. Nonanal can reach slightly higher values, since in one of the wines  $3.3 \mu\text{g l}^{-1}$  of nonanal were measured, being its perception threshold  $1 \mu\text{g l}^{-1}$  [31]. Unlike the previous cases, decanal reaches its maximum concentration in Chardonnay wines ( $1.2 \mu\text{g l}^{-1}$ ). In addition, this component could be particularly active, since its threshold value is only  $0.1 \mu\text{g l}^{-1}$  [31]. The levels of (*E*)-2-nonenal were clearly higher in red wines, particularly in the most aged. However, the maximum level found ( $0.47 \mu\text{g l}^{-1}$ ) is

Table 4  
Matrix effects

	Added ( $\mu\text{g l}^{-1}$ )	Red wine 1 (%)	Red wine 2 (%)	White wine (%)
Octanal	1	64 ± 6	63 ± 3	60 ± 7
Nonanal	1.4	105 ± 8	101 ± 6	94 ± 9
Decanal	1.5	109 ± 10	104 ± 9	100 ± 12
( <i>E</i> )-2-Nonenal	0.2	91 ± 5	93 ± 6	93 ± 4
( <i>E, Z</i> )-2,6-Nonadienal	0.2	91 ± 12	95 ± 8	99 ± 13

Increment of the signal measured in the analysis of spiked samples, given as (%) of the signal produced by the spiked amount directly placed on the cartridge.

Table 5  
Wine analysis

	Chardonnay			Merlot			Aged wines		
	Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.
Octanal	0.20	0.46	<LD	0.57	0.97	0.37	0.24	0.27	0.23
Nonanal	0.95	2.4	<LD	1.85	3.3	1.47	0.72	0.83	0.68
Decanal	0.79	1.25	<LD	0.76	1.00	0.65	0.54	0.75	0.41
( <i>E</i> )-2-Nonenal	0.031	0.092	<LD	0.23	0.37	0.036	0.32	0.47	0.20

Mean, maximum and minimum aldehyde levels ( $\mu\text{g l}^{-1}$ ) in three different groups of six wines.

far below those documented by Chatonnet and Dubourdieu [10], in agreement with the absence of aromatic defects in the wines considered in the present study. Nevertheless, the low threshold value for (*E*)-2-nonenal ( $68 \text{ ng l}^{-1}$ ) [10] indicates that this is a component whose concentration is sufficient to make it aromatically active.

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### References

- [1] H. Maarse, Volatile Compounds in Foods and Beverages, Marcel Dekker, New York, NY, 1991.
- [2] M. Meilgaard, A. Elizondo, E. Moya, *Tech. Q.* 7 (1970) 143.
- [3] P.W. Meijboom, G.A. Jongenotter, *J. Am. Oil Chem. Soc.* 58 (1981) 680.
- [4] C.V. Ranson, H.D. Belitz, *Z. Lebens. Unters. Forsch.* 195 (1992) 515.
- [5] C.V. Ranson, H.D. Belitz, *Z. Lebens. Unters. Forsch.* 195 (1992) 523.
- [6] M. Laska, P. Teubner, *Chem. Senses* 24 (1999) 263.
- [7] C.D. Azzara, L.B. Campbell, in: G. Charalambous (Ed.), *Off-Flavors in Foods and Beverages*, Elsevier, Amsterdam, 1992, p. 329.
- [8] L.C. Verhagen, in: J.R. Piggott, A. Paterson (Eds.), *Understanding Natural Flavors*, Blackie Academic & Professional, London, 1994, p. 211.
- [9] P. Chatonnet, in: A.L. Waterhouse, S.E. Ebeler (Eds.), *Chemistry of Wine Flavor*, American Chemical Society, Washington, DC, 1998, p. 180.
- [10] P. Chatonnet, D. Dubourdieu, *J. Sci. Food Agric.* 76 (1998) 179.
- [11] A. Escudero, E. Asensio, J. Cacho, V. Ferreira, *Food Chem.* 77 (2002) 325.
- [12] H. Maarse, C.A. Vischer, *Volatile Compounds in Food. Alcoholic Beverages. Qualitative and Quantitative Data*, TNO-CIVO, Food Analysis Institute, AJ Zeist, The Netherlands, 1989.
- [13] J.C. Cabanis, M.T. Cabanis, V. Cheynier, P.L. Teissedre, in: C. Flanzy (Ed.), *Oenologie, Fondements Scientifiques et Technologiques*, Lavoisier, Paris, 1998, p. 318.
- [14] R. Flamini, G. De Luca, R. Di Stefano, *Vitis* 41 (2002) 107.
- [15] S.E. Ebeler, R.S. Spaulding, in: A.L. Waterhouse, S.E. Ebeler (Eds.), *Chemistry of Wine Flavor*, American Chemical Society, Washington, DC, 1998, p. 166.
- [16] M.N. Lau, J.D. Ebeler, S.E. Ebeler, *Am. J. Enol. Vitic.* 50 (1999) 324.
- [17] G. De Revel, A. Bertrand, *J. Sci. Food Agric.* 61 (1993) 267.
- [18] J.P. Vidal, S. Estreguil, R. Cantagrel, *Chromatographia* 36 (1993) 183.
- [19] W. Wardencki, P. Sowinski, J. Curylo, *J. Chromatogr. A* 984 (2003) 89.
- [20] P.A. Martos, J. Pawliszyn, *Anal. Chem.* 70 (1998) 2311.
- [21] P.A. Martos, J. Pawliszyn, in: C.J. Mussinan, M.J. Morello (Eds.), *Flavor Analysis. Developments in Isolation and Characterization*, American Chemical Society, Washington, DC, 1998, p. 92.
- [22] D. Grosjean, K. Fung, *Anal. Chem.* 54 (1982) 1221.
- [23] W. Wardencki, J. Orlita, J. Namiesnik, *Fresenius J. Anal. Chem.* 369 (2001) 661.
- [24] E.E. Stashenko, M.A. Puertas, W. Salgar, W. Delgado, J.R. Martínez, *J. Chromatogr. A* 886 (2000) 175.
- [25] C. Ortega, R. López, J. Cacho, V. Ferreira, *J. Chromatogr. A* 931 (2001) 31.
- [26] R. López, M. Aznar, J. Cacho, V. Ferreira, *J. Chromatogr. A* 966 (2002) 166.
- [27] EPA Method 556, Environmental Monitoring and System Laboratory, Cincinnati, OH, USA, 1998.
- [28] V. Ferreira, R. López, M. Aznar, in: J. Jackson (Ed.), *Analysis of Taste and Aroma*, Springer-Verlag, Berlin, 2001, p. 89.
- [29] P. Lövkvist, J.A. Jönsson, *Anal. Chem.* 59 (1987) 818–821.
- [30] V. Ferreira, I. Jarauta, L. Ortega, J. Cacho, *J. Chromatogr. A* 1025 (2004) 147.
- [31] J.C. Leffingwell, <http://www.leffingwell.com/burnt.htm>, 2002.