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ARTICLE

A New Method for the Determination of Carbonyl Compounds in Wines by Headspace Solid-Phase Microextraction Coupled to Gas Chromatography−Ion Trap Mass Spectrometry

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A new analytical method for the determination of 18 carbonyl compounds [2,3-pentadione, hexanal, (*E*)-2-hexen-1-al, octanal, acetoin, (*E*)-2-octenal, furfural, decanal, (*E*)-2-nonenal, benzaldehyde, 5-methylfurfural, (*E*,*E*)-2-*cis*-6-nonadienal, β-damascenone, phenylacetaldehyde, acetophenone, (*E*,*E*)-2,4-decadienal, benzophenone, and vanillin] in wines using automated headspace solid-phase microextraction (HS/SPME) coupled to gas chromatography−ion trap mass spectrometry (GC−ITMS) was developed. Five fibers with different polarities were tested, and a study of the influence of various factors such as time and extraction temperature, desorption time and temperature, pH, and ionic strength and content in tannins, anthocyans, sucrose, SO_2, and alcoholic degree was conducted. These factors were optimized using a synthetic wine doped with the different analytes. The proposed method affords wide ranges of linearity, good linearity ($r^2 > 0.998$), values of repeatability and reproducibility lower than 5.5% of RSD, and detection limits ranging from 0.62 μg/L for β-damascenone to 129.2 μg/L for acetoin. Therefore, the optimized method was applied to the quantitative analysis of the aforementioned analytes in real samples of wines.

KEYWORDS: Carbonyl compounds; wines; solid-phase microextraction; gas chromatography−ion trap mass spectrometry

INTRODUCTION

A large number of carbonyl compounds (aldehydes and ketones) have been detected in wines (*1*). Very few aldehydes appear to come from the grapes, most of them being generated during fermentation, processing, or aging in wood as a result of oxidation of alcohols (*2*). Aldehydes are also produced by decarboxylation of the corresponding keto acid arising as an intermediate in the metabolism of amino acids. On the other hand, hexanal, hexenals, and (*E*,*E*)-2,4-decadienal are formed from the enzymatic oxidation of linoleic and linolenic acids during grape pressing. Ketones, however, may have different origins: few of them are found in grapes, but they normally overcome the fermentation and remain in the wine. Others such as acetoin and 2,3-pentadione are produced during alcoholic fermentation, while acetoin may also be produced during malolactic fermentation as a result of bacterial degradation of citric acid. Furfural derivatives are produced by carbohydrate dehydration and cyclization during ripening. Some ketones such as β-damascenone may come from the degradation of carotenoids (*1*). Vanillin originates from oak lignin, either during pyrolysis, which extracts it quickly, or by hydrolysis or oxidation, which extracts it more slowly (*3*).

The production of carbonyl compounds depends on factors such as fermentation temperature, SO_2 content, yeast type, pH, and oxygenation of the medium (*4*). The greater part of the carbonyl compounds found in wine are at low concentrations, < 1 mg/L. However, from the sensory point of view, some carbonyl compounds may contribute to the wine aroma with green notes (*5*). Baro and co-authors (*6*) highlight the importance of aldehydes in the development of wine aroma as well as its deterioration. Many carbonyl compounds such as *trans*-2-octenal, *trans*-2-nonenal, hexanal, benzaldehyde, furfural, and 5-methylfurfural originate in the oxidation process of wine by the action of oxygen and can contribute to wine aroma with unpleasant notes (*7*). The hexanal and hexenal aldehydes are, in large part, responsible for herbaceous and "grassy" aromas that are associated with wines made with unripe grapes. Phenylacetaldehyde, 3-methylbutanal, and (*E*)-2-octenal are identified as potential contributors to wine aroma (*8*), phenylacetaldehyde also being an important contributor to the aroma of botrytized wines (*9*) and Muscat wines (*10*). Furfural evokes in some wines cooking aromas and is considered to be an indicator of time of storage, as its content increases with increasing time in the bottle (*11*, *12*). Vanillin is associated with conservation in wooden barrels, giving the wines a pronounced vanilla aroma and being widely assumed to contribute to the aroma of alcoholic drinks aged in wood (*13*). β-Damascenone has a strong relationship with the characteristic aromas of certain grape varieties such as Chardonnay (*14*), Riesling (*15*), and Garnacha (*16*, *17*). On the other hand, acetoin, among other compounds, is considered to be an important odorant in sparkling wine (*18*), while acetophenone and β-damascenone are counted among the major sensory compounds in Italian white wines (*19*).

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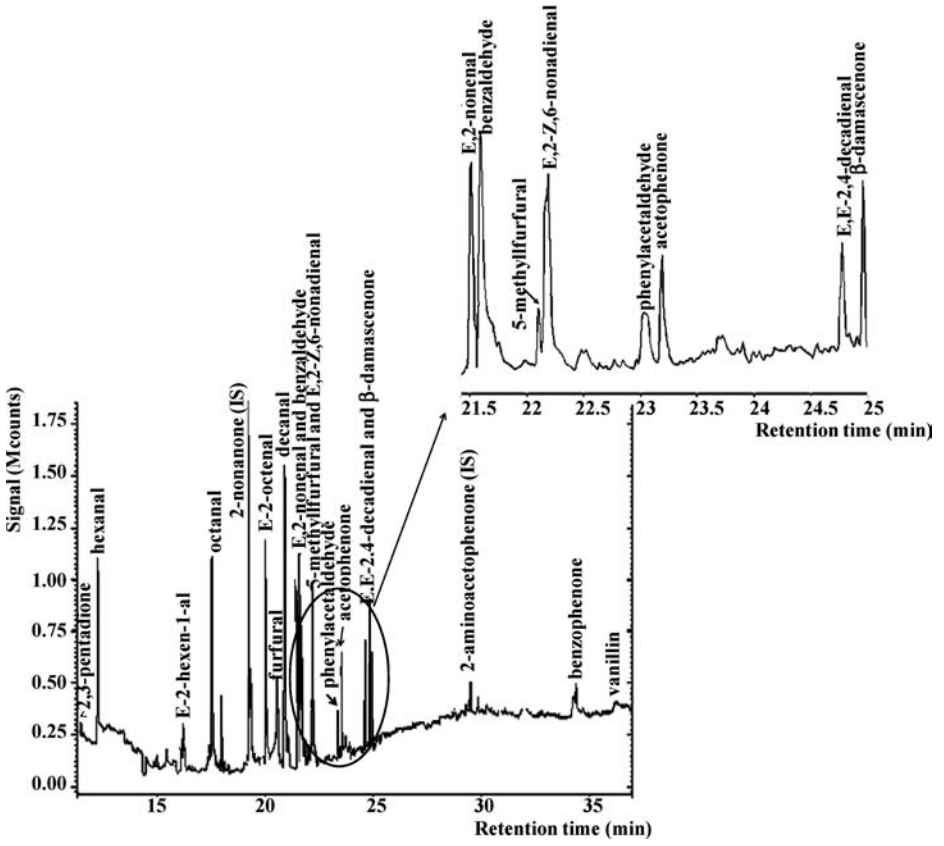

Figure 1. Chromatogram obtained with a DVB/CAR/PDMS fiber (total ion counts vs retention time) for a synthetic wine spiked with the different analytes.

Table 1. Retention Times and Quantification Ions for the Different Analytes

compound	Q ions (*m*/*z*)	retention time (min)
2,3-pentadione	43 + 57	11.51
hexanal	44 + 56 + 57	12.39
(*E*)-2-hexen-1-al	41 + 55 + 69 + 83 + 98	16.28
octanal	42 + 44 + 55 + 56 + 57 + 69 + 84	17.63
acetoin	45	17.71
2-nonanone (IS)	71	19.33
(*E*)-2-octenal	41 + 55 + 70 + 83	20.07
furfural	95 + 96	20.63
decanal	41 + 43 + 44 + 57 + 70 + 82	20.94
(*E*)-2-nonenal	41 + 43 + 55 + 70 + 83	21.54
benzaldehyde	77 + 105 + 106	21.62
5-methylfurfural	109 + 110	22.12
(*E*)-2-(*Z*)-6-nonadienal	41 + 69 + 70	22.21
phenylacetaldehyde	91	23.08
acetophenone	51 + 77 + 105 + 120	23.21
(*E*,*E*)-2,4-decadienal	81	24.79
β-damascenone	69 + 121 + 190	24.98
2-aminoacetophenone (IS)	65 + 92 + 120 + 135	29.55
benzophenone	51 + 77 + 105 + 182	34.43
vanillin	81 + 109 + 106 + 151 + 152	36.01

Carbonyl compounds have been analyzed in different matrices such as water, the environment, foods, pharmaceutical products, biological samples, etc., by means of HPLC with mass spectrometry (MS), ultraviolet (UV), or diode array detectors (DAD) and, mainly GC coupled to MS, electron capture (ECD) or flame ionization (FID) detectors (*20*−*24*). Although the literature devoted to alcoholic matrices is scant, carbonyl compounds have been analyzed in whiskey (*25*), alcoholic drinks derived from cane sugar (*26*), cognac and calvados (*27*), beers (*28*), and wines (*29*, *30*). In general, these methods require a derivatization process combined with a concentration step such as liquid−liquid extraction or solid-phase extraction that is tedious and time-consuming and involves solvents.

Headspace solid-phase microextraction (HS/SPME) is a simple, fast, sensitive, and solvent-free extraction technique that allows the extraction and concentration steps to be performed simultaneously. Several papers have appeared using derivatization of some carbonyl compounds followed by extraction of the derivative products by HS/SPME in different matrices (*31*−*35*). Although SPME has been applied to the determination of different chemical compounds in wine (*36*−*41*), there are few papers that report on the direct determination of carbonyl compounds using SPME without prior derivatization.

The aim of this work is to develop an analytical method for direct determination of 18 carbonyl compounds using the headspace solid-phase microextraction technique coupled with gas chromatography with an ion trap mass spectrometry detector (HS/SPME−GC−ITMS) and to assess the possibilities of its application to determinations in real samples of wine.

EXPERIMENTAL PROCEDURES

Chemicals and Reagents. The following compounds were studied (abbreviations in parentheses) (CAS number in brackets): 2.3-pentadione [600-14-6], (*E*)-2-octenal [2548-87-0], (*E*)-2-nonenal [18829-56-6], (*E*)-2-(*Z*)-6-nonadienal [557-48-2], phenylacetaldehyde [122-78-1], vanillin [121-33-5], and 2-aminoacetophenone (IS) [613-89-8] supplied by Aldrich (Steinheim, Germany, and Milwaukee, WI); hexanal [66-25-1], (*E*)-2-hexen-1-al [6728-26-3], octanal [124-13-0], furfural [98-01-1], decanal [112-31-2], benzaldehyde [100-52-7], 5-methylfurfural [620-02-0], acetophenone [98-86-2], (*E*,*E*)-2,4-decadienal [25152-84-5], benzophenone [119-61-9], acetoin [513-86-0], and 2-nonanone (IS) [821-55-6] supplied by Fluka (Buchs, Switzerland); and β-damascenone [23726-93-4] supplied by Firmenich. These standards were supplied with purity above 99%. Sodium chloride [7647-14-5] (Merck, Darmstadt, Germany) was used to control ionic strength. L-(+)-Tartaric acid [87-69-4] (Merck) was used to prepare

Table 2. Sensitivity Order of Fibers for the Different Analytes and Extraction Times To Reach Equilibrium in Parentheses (minutes)

compound/fiber	MW	first	second	third	fourth	fifth
acetoin	88.11	CAR/PDMS (120)	PA (60) ≈ DVB/CAR/PDMS (90)		PDMS/DVB (60)	PDMS/100 (15)
furfural	96.09	CAR/PDMS (≥150)	DVB/CAR/PDMS (120)	PDMS/DVB (120)	PA (60)	PDMS/100 (30)
(*E*)-2-hexen-1-al	98.15	CAR/PDMS (90)	DVB/CAR/PDMS (60)	PDMS/DVB (15)	PA (30)	PDMS/100 (15)
2,3-pentadione	100.12	CAR/PDMS (90)	DVB/CAR/PDMS (45)	PDMS/DVB (60)	PA (45)	PDMS/100 (30)
hexanal	100.16	CAR/PDMS (90)	DVB/CAR/PDMS (60)	PDMS/DVB (60)	PA (45)	PDMS/100 (5)
benzaldehyde	106.12	CAR/PDMS (≥150)	DVB/CAR/PDMS (120)	PDMS/DVB (120)	PA (45)	PDMS/100 (15)
5-methylfurfural	110.11	CAR/PDMS (≥150)	DVB/CAR/PDMS (≥150)	PDMS/DVB (60)	PA (30)	PDMS/100 (5)
phenylacetaldehyde	120.15	DVB/CAR/PDMS (120)	PDMS/DVB (120)	CAR/PDMS (120)	PA (60)	PDMS/100 (30)
acetophenone	120.15	DVB/CAR/PDMS (120)	CAR/PDMS (120)	PDMS/DVB (120)	PA (60)	PDMS/100 (15)
(*E*)-2-octenal	126.20	DVB/CAR/PDMS (120)	PDMS/DVB (120)	PA (60) ≈ CAR/PDMS (90)		PDMS/100 (15)
octanal	128.22	DVB/CAR/PDMS (120)	PDMS/DVB (30)	PA (45)	PDMS/100 (15)	CAR/PDMS (120)
2-aminoacetophenone (IS)	135.17	DVB/CAR/PDMS (≥150)	PDMS/DVB (150) ≈ PA (120)		CAR/PDMS (120)	PDMS/100 (45)
(*E*)-2-(*Z*)-6-nonadienal	138.21	DVB/CAR/PDMS (120)	PDMS/DVB (120)	PA (90)	PDMS/100 (60)	CAR/PDMS (120)
(*E*)-2-nonenal	140.23	PDMS/DVB (120)	DVB/CAR/PDMS (120)	PA (90)	PDMS/100 (60)	CAR/PDMS (120)
2-nonanone (IS)	142.24	DVB/CAR/PDMS (120)	PDMS/DVB (120)	PA (60) ≈ PDMS/100 (30)	CAR/PDMS (120)	
vanillin	152.15	PA (60)	DVB/CAR/PDMS (120)	PDMS/100 (≥150)	PDMS/DVB (≥150)	CAR/PDMS (90)
(*E*,*E*)-2,4-decadienal	152.24	PDMS/DVB (120)	PA (90)	DVB/CAR/PDMS (120) ≈ PDMS/100 (60)		CAR/PDMS (120)
decanal	156.27	DVB/CAR/PDMS (120) ≈ PDMS/DVB (120)		PA (45)	PDMS/100 (45)	CAR/PDMS (90)
benzophenone	182.22	PA (≥150) ≈ PDMS/DVB (≥150) ≈ PDMS/100 (≥150)			DVB/CAR/PDMS (120)	CAR/PDMS (120)
β-damascenone	190.28	DVB/CAR/PDMS (120)	PDMS/DVB (120)	PA (90) ≈ PDMS/100 (90)		CAR/PDMS (120)

the synthetic wine. Sodium hydroxide [1310-73-2] (Panreac, Barcelona, Spain) was used to adjust the pH. Tannins and anthocyans (Agrovin S.A., Ciudad Real, Spain) were used to study the influence of the polyphenols. Saccharose [57-50-1] (Panreac) was used to study the influence of the sugar content. Potassium metabisulfite [16731-55-8] (Merck) was used to study the influence of the sulfur dioxide. Absolute ethanol [64-17-5] (Merck) and MQ-water (Millipore, Bedford, MA) were used as solvents.

Standard solutions of the different analytes (≈100 mg/L), with the exception of acetoin (5036 mg/L), were prepared in ethanol. A standard solution of 2-aminoacetophenone (3.82 mg/L) and 2-nonanone (4.03 mg/L), used as internal standards, was prepared in 13% ethanol. Likewise, a solution of potassium metabisulfite (5.55 g/L) for studying the influence of SO_2 was prepared in ethanol, and a solution of tannins (25.39 g/L) and anthocyans (127.00 g/L) was prepared in water. All these solutions were stored at 5 °C.

Working solutions were prepared from standard solutions by dilution.

Equipment. Class A volumetric flasks, vials of 2 mL, Gilson pipetmans regularly verified for precision and accuracy, a precision balance (Sartorius BP 210-S), a pH meter (WTW, pH 197-S), Milli Q-gradient A10 (Millipore), and a mechanical shaker (Selecta, Rotabit) were used to prepare solutions.

SPME Fibers. The fibers used (Supelco, Bellefonte, PA) were coated with different stationary phases and various film thicknesses: polydimethylsiloxane of 100 μm (PDMS/100), carboxen/polydimethylsiloxane of 75 μm (CAR/PDMS), polydimethylsiloxane/divinylbenzene of 65 μm (PDMS/DVB), polyacrylate of 85 μm (PA), and divinylbenzene/carboxen/polydimethylsiloxane of 50 and 30 μm (DVB/CAR/PDMS). They were conditioned before use by being inserted the GC injector under the following conditions: 250 °C for 0.5 h for PDMS/100, 300 °C for 1 h for CAR/PDMS, 250 °C for 0.5 h for PDMS/DVB, 300 °C for 2 h for PA, and 270 °C for 1 h for DVB/CAR/PDMS.

Chromatography. The analyses were conducted on a 3800 GC gas chromatograph equipped with an 8200 Standalone autosampler, a 1079 split/splitless injector, and a Saturn 2000 mass spectrometry detector (Varian, Walnut Creek, CA). The injection was made in splitless mode, using a linear 0.75 mm inside diameter that improved the GC resolution. The temperature of the detector was 300 °C, using electronic impact (EI) as the ionization mode.

The separations were performed using a DB-WAXETR capillary column [60 m × 0.25 mm (inside diameter), 0.5 μm film thickness] (J&W Scientific) with an injector temperature of 250 °C (valid for all the fibers) and the following oven temperature program: 60 °C (10 min), rate of 11 °C/min, 240 °C until 38 min of chromatogram. The carrier gas was helium with a flow rate of 2 mL/min.

Figure 1 shows the chromatogram of a synthetic wine spiked with all the compounds where a good separation and resolution among the different peaks can be seen.

Each compound was identified using the spectra obtained with standard compounds from the NIST library. Retention times and quantification ions of each compound are listed in **Table 1**.

Solid-Phase Microextraction Procedure. For the optimization of instrumental and experimental parameters, a synthetic model wine solution doped with the analytes being studied was used: 5.5 g/L tartaric acid, 13% by volume, pH 3.2, and a standard concentration of 0.10 mg/L for all the analytes except acetoin (4.03 mg/L).

To prepare solutions, 0.77 mL of the spiked synthetic wine, we added 0.24 g of NaCl, and 30 μL of the internal standard solution to a 2 mL headspace vial. The vial was tightly capped with a PTFE-lined cap and then shaken for 10 min at 200 rpm.

Calibration Procedure. Eight standard solutions of the different analytes at increasing concentrations were prepared in a model synthetic wine: pH 3.2, 5.5 g/L tartaric acid, and 13% by volume. The calibration was developed using 2 mL vials, to which were added 0.24 g of NaCl, 0.77 mL of the synthetic spiked wine sample, and 30 μL of the internal standard solution. Vials were capped with a PTFE-lined cap and shaken for 10 min at 200 rpm.

Procedure for Analysis of Real Samples. The analysis of samples was conducted via addition of 0.24 g of NaCl, 0.77 mL of the wine sample, and 30 μL of the internal standard solution to a 2 mL vial capped with a PTFE-lined cap, and then the vial was shaken for 10 min at 200 rpm.

All studies were conducted in triplicate and average values calculated.

RESULTS AND DISCUSSION

Method Development. With the aim of optimizing the extraction of the different carbonyl compounds, we have studied the influence of five different fibers: polyacrylate (PA), carboxen/polydimethylsiloxane (CAR/PDMS), divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS), polydimethylsiloxane of 100 μm (PDMS/100), and polydimethylsiloxane/divinylbenzene (PDMS/DVB) [extraction time to 150 min (5, 15, 30, 45, 60, 90, 120, and 150 min)] in a synthetic wine doped with the different analytes. These extraction studies were performed in 25 °C thermostated vials, with stirring, with a saturated solution of sodium chloride and the fiber in the headspace.

Acetoin was the compound with the lower normalized peak area (absolute peak area/analyte concentration) in all the fibers followed by 2-aminoacetophenone. Most analytes reached extraction equilibrium in the different fibers with the exception of furfural, benzaldehyde, and 2-aminoacetophenone in the CAR/PDMS fiber, 5-methylfurfural in CAR/PDMS and DVB/CAR/PDMS fibers, vanillin in PDMS/100 and PDMS/DVB fibers, and benzophenone in PA, PDMS/DVB, and PDMS/100 fibers.

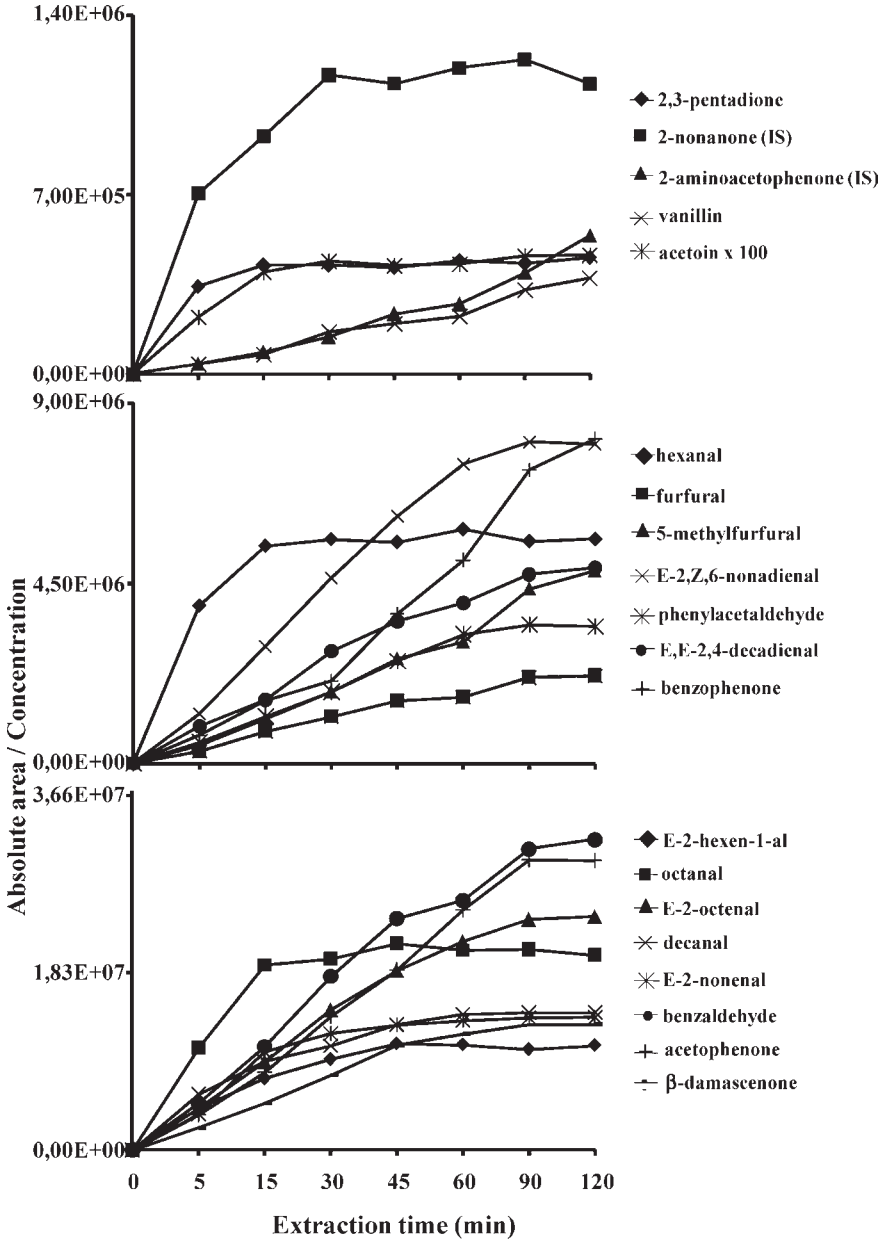

Figure 2. Extraction profiles of the different analytes vs extraction time for a DVB/CAR/PDMS fiber at 40 °C.

In **Table 2**, we present a summary of the information obtained in this study that includes the decreasing order of sensitivity of the five fibers (from first to fifth) for the different carbonyl compounds and the time needed to reach extraction equilibrium (in minutes) for each compound in every fiber considering the normalized peak areas of the different carbonyl compounds.

As one can see, the fibers with higher normalized peak areas are CAR/PDMS, for lower-molecular weight analytes (MW < 115), and DVB/CAR/PDMS, in general, for higher-molecular weight analytes. By contrast, the fibers with lower normalized peak areas are PDMS/100, for smaller analytes (MW < 127), and CAR/PDMS, for larger analytes (MW > 150). Likewise, it should be noted that, in general, analytes reach extraction equilibrium faster in the absorption fibers (< 60 min) than in the adsorption fibers (≈120 min).

Comparing the two absorptive fibers (PDMS and PA), we find that the PA fiber shows higher values of normalized peak areas for most of the analytes, which could be explained by the fact that the PA fiber, because of its polar nature, attracts the carbonyl compounds more in the headspace, being also polar in nature, rather than the PDMS fiber, which is nonpolar in nature. Moreover, the PDMS/100 fiber is the least sensitive of all the fibers for 12 of the 20 studied compounds, particularly for those with lower molecular weights (which are more polar). On the other hand, the majority of analytes reach extraction equilibrium faster in the PDMS/100 fiber than in the PA fiber. Likewise, both fibers reach extraction equilibrium more quickly with lower-molecular weight analytes (MW < 130).

Comparing the three adsorption fibers CAR/PDMS, PDMS/DVB, and DVB/CAR/PDMS, we find that the CAR/PDMS fiber is the one with the greatest extraction efficiency for the lower-molecular weight analytes (MW < 115) and a lower efficiency for the higher-molecular weight compounds. This may be due to the fact that the CAR/PDMS fiber has a higher proportion of micropores, which makes it more suited to the lower-molecular weight analytes, but is less effective with those with increasing molecular weights. Thus, this fiber is the best one for seven and the worst of all for eight of the 20 analytes studied. On the other hand, the DVB/CAR/PDMS fiber generally affords a higher extraction efficiency than the PDMS/DVB fiber, which may be

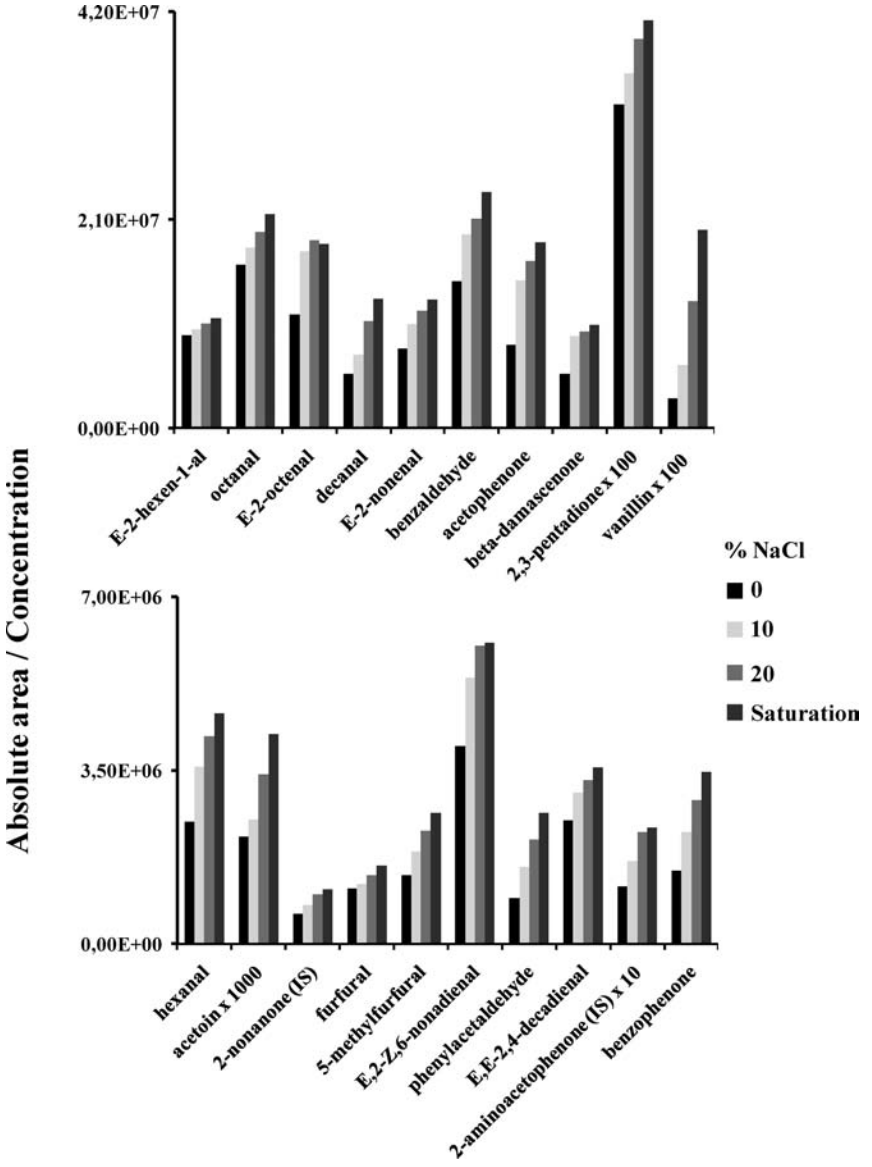

Figure 3. Influence of ionic strength on the extraction of the different analytes on a DVB/CAR/PDMS fiber.

due to the fact that in the 65 μm PDMS/DVB fiber meso- and macropores predominate, with a lower efficiency for smaller analytes, while the DVB/CAR/PDMS fiber that combines two layers, one of PDMS/DVB of 50 μm and another of CAR/PDMS of 30 μm, has a larger number of micropores, which makes it more effective for lower-molecular weight analytes than the PDMS/DVB fiber. The increased sensitivity of the DVB/CAR/PDMS fiber should also be related to its greater thickness (50 + 30 μm) compared to that of the PDMS/DVB fiber (65 μm). Taking all the above into account, we have chosen the DVB/CAR/PDMS fiber as the best for further studies of the carbonyl compounds.

Because in the DVB/CAR/PDMS fiber most analytes reached extraction equilibrium at 120 min, and some did not even reach equilibrium during the study period of 150 min, it was considered desirable to perform another extraction study with this fiber at a higher extraction temperature. The selected temperature was 40 °C, and an extraction time of up to 120 min was tested (5, 15, 30, 45, 60, 90, and 120 min). **Figure 2** shows the extraction curves for the triple fiber at 40 °C as a function of extraction time. The obtained results show that with an increase in the temperature to 40 °C, the analytes in general reach extraction equilibrium faster and an increase in the normalized peak area is observed for all analytes, with the exceptions of 2,3-pentadione, hexanal, acetoin, and vanillin.

In view of these results, 40 °C was selected as the extraction temperature and 45 min as the extraction time, and although some analytes do not reach equilibrium within that time, this is not a disadvantage to quantify if the extraction time and stirring speed of the samples are kept constant, as in our case.

To optimize desorption, we studied the influence of temperature desorption at two temperatures (250 and 270 °C) and desorption time at three different times (2, 5, and 10 min), under the extraction conditions selected (40 °C and 45 min). After each sample injection, a blank puncture was performed to confirm whether desorption was complete. The results show that the normalized peak areas of all analytes increase with temperature and desorption time, those areas being normalized similarly at 10 min for both temperatures, which would indicate that analyte desorption was complete at 10 min. This was ratified by the blank puncture that showed no response for the different analytes. Therefore, 250 °C and 10 min have been selected as the desorption conditions.

The addition of salt to samples causes an increase in ionic strength, facilitating transfer to the headspace in the case of polar

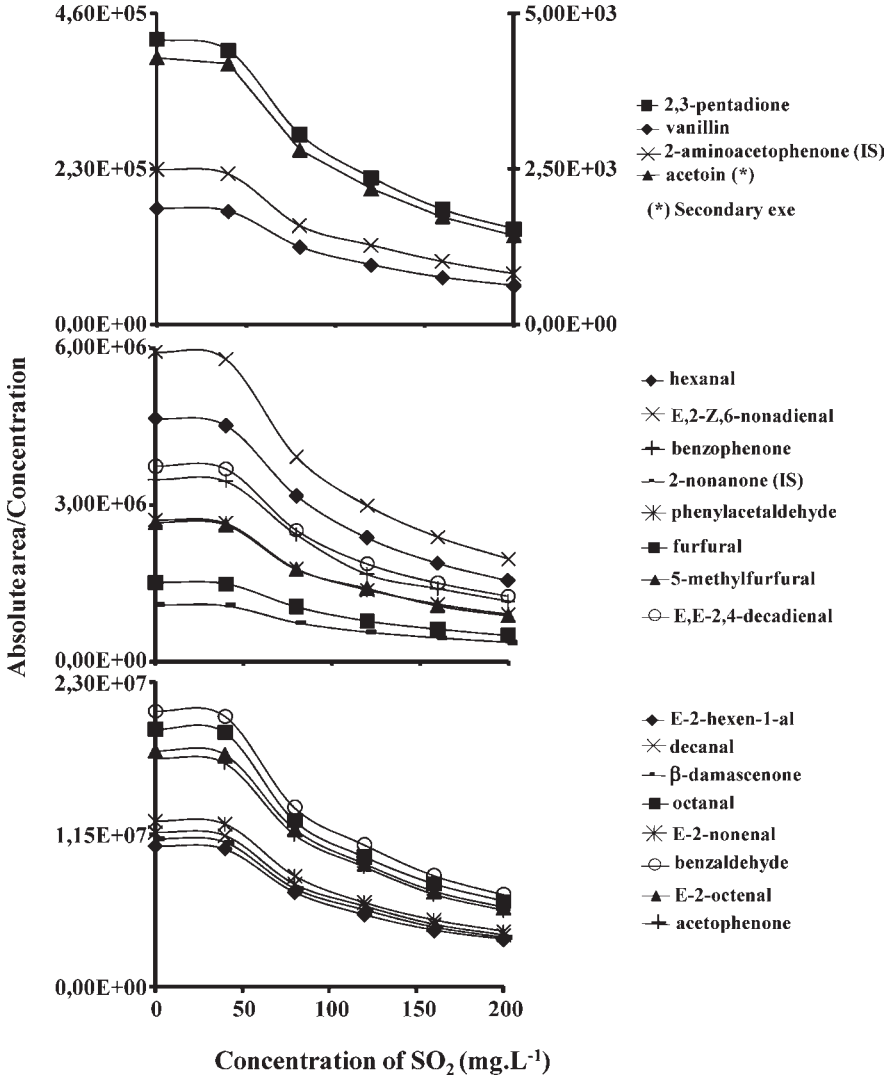

Figure 4. Normalized peak areas of the different analytes vs sulfur dioxide concentration on a DVB/CAR/PDMS fiber.

compounds. To study its influence on analyte extraction, we chose sodium chloride as the salt and its concentration was varied from zero to saturation. The results obtained, in values of normalized peak areas, for the different analytes are shown in **Figure 3**. As one can see, in general, an increase in ionic strength increases their level of extraction in all cases; we, therefore, selected to work under conditions of saturation in sodium chloride in further studies.

Usually sulfur dioxide (SO_2) is added to wine, among other reasons, as an antioxidant and antiseptic agent, to protect from the action of atmospheric oxygen and microorganisms that degrade the wine. The sulfur reacts with those compounds having carbonyl groups in their structure to form the so-called combined sulfur, thus altering the concentration of these compounds in the free state. Because the analytes under study have carbonyl groups in their structures, we proceeded to study the influence of the concentration of SO_2 (0−200 mg/L) on analyte extraction. The results are presented in **Figure 4**. One can see that between 0 and 40 mg/L SO_2 there is very little variation in the value of the normalized peak areas for the different analytes; between 40 and 80 mg/L, a significant decline takes place, continuing from 80 to 200 mg/L, although more slowly. It is noteworthy that the internal standards, 2-nonanone and 2-aminoacetophenone, follow the same pattern of behavior as the other carbonyl compounds, such that in the calculation of the relative areas of the different analytes with respect to the internal standard they are independent of the concentration of sulfur dioxide. The tests conducted with other internal standards that do not have carbonyl groups in their structures have not been successful in correcting the effect of changes in the areas of the analytes via variation of the concentration of sulfur dioxide.

The ethanol content influences analyte extraction because it competes with them for the active sites of the fiber, such that the higher the alcohol content of wine, the fewer active sites will be available to retain analytes (*37*). With the aim of determining the influence of the level of ethanol on analyte extraction, we performed a study of the extraction efficiency by varying the ethanol content of a synthetic wine solution between 11 and 14%. **Figure 5** shows the variation of normalized peak areas of the different analytes on the basis of the alcohol content. One can see that increasing the alcohol content decreases the peak area value of the different analytes, including the two internal standards.

To determine if the internal standards corrected the influence of alcoholic content, we proceeded to calculate the relative areas of the different analytes with respect to both internal standards for the different percentages of ethanol. The obtained results indicated that 2-nonanone better corrected the influence of the ethanol content in the case of 2,3-pentadione, hexanal, (*E*)-2-hexen-1-al, acetoin, furfural, (*E*)-2-nonenal, 5-methylfurfural, (*E*,*E*)-2,4-decadienal, benzophenone, and vanillin compounds,

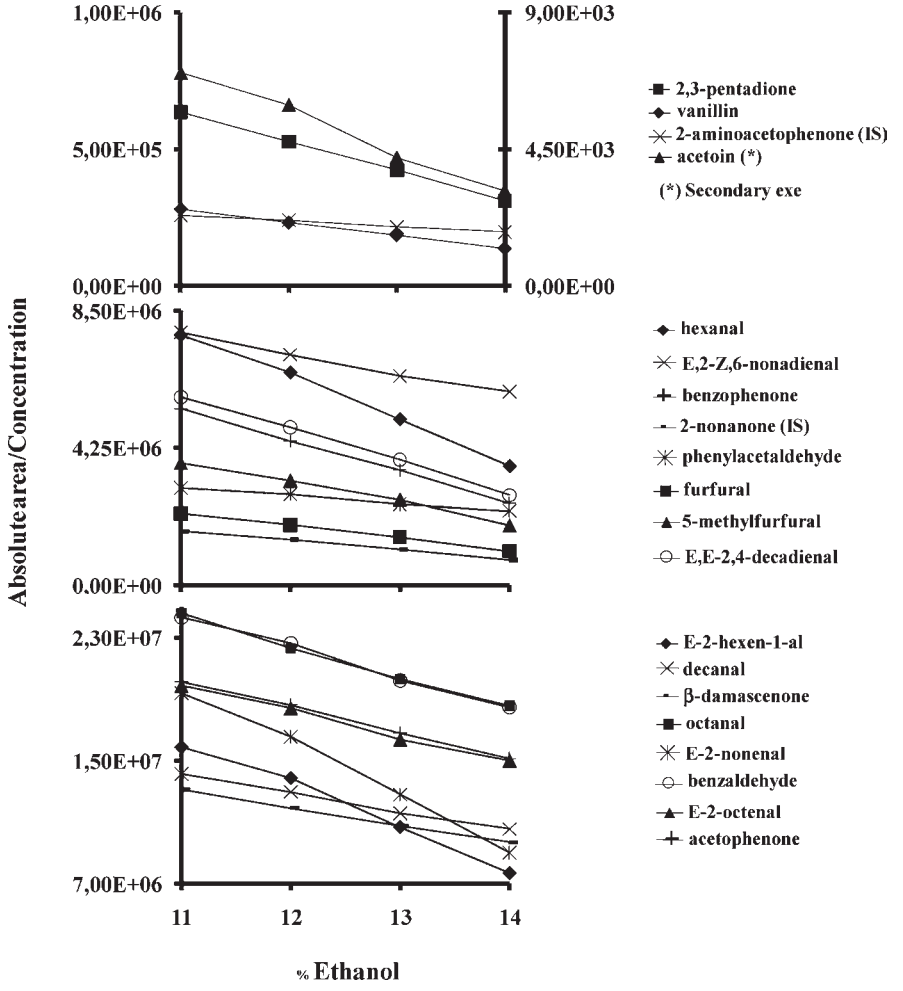

Figure 5. Normalized peak areas of the different analytes vs alcoholic content on a DVB/CAR/PDMS fiber.

and 2-aminoacetophenone in the remainding analytes. We then used the internal standard that best corrected the influence of the level of ethanol to calculate the relative areas of the different analytes.

We also studied the influence of other parameters such as pH between 3 and 4, tannins at concentrations from 0 to 1 g/L, anthocyans at concentrations between 0 and 5 g/L, and sugars at concentrations from 0 to 200 g/L, which often vary depending on the type of wine analyzed, finding that none of them influences the extraction of analytes.

Method Validation. The calibrated solutions were prepared in 2 mL vials, with 13% ethanol, 0.77 mL of the synthetic wine sample, 30 μL of the internal standard solution (0.80 mL of the liquid phase), saturated in NaCl, stirring, fiber in headspace, a 40 °C extraction temperature, and a 45 min extraction time. Eight levels of increased concentrations were prepared for all the analytes. The concentration ranges were selected according to the concentration of these compounds in wines. The range of concentrations studied, limit of detection, intercept and slope, coefficient of regression, repeatability, and reproducibility for every compound are listed in **Table 3**. Limits of detection were determined as 3 times the noise of five blank injections. As one can see, the detection limits are low, a few micrograms per liter, for all the compounds with the exception of acetoin (129 μg/L), although this compound is present in wines in amounts of approximately milligrams per liter. A linear regression analysis of relative peak areas referred to the corresponding internal standard versus the analyte concentration was performed. The application of a lack of fit test showed that the calculated F ratio was not significant for all compounds. The values of the correlation coefficients (R^2) were 0.998 for acetoin and 0.999 for the rest of the carbonyl compounds.

The repeatability and reproducibility were estimated by the relative standard deviation (RSD) of the area relative to the selected internal standard for five consecutive solutions and three different days. All the values obtained were lower than 6%, ranging from 1.4% for (*E*)-2-nonenal to 5.5% for 2,3-pentadione.

Matrix Effect. Considering that the real samples of wine are more complex than synthetic wines, a recovery study was performed. For this purpose, known quantities of the different analytes at two concentration levels were added to different samples of red and white wines. The results of the recoveries for the different analytes are listed in **Table 4**. As one can see, the majority of the analytes showed a matrix effect. The matrix effect varies from 20% for (*E*)-2-octenal in red wine to 190% for acetoin in red wines and vanillin in both types of wine. Only benzophenone exhibited no matrix effect in both types of wines. Furfural and β-damascenone had no matrix effect on white wines, while hexanal, octanal, and (*E*,*E*)-2,4-decadienal showed no matrix effect on red wines. The rest of the analytes showed a matrix effect in both white and red wines. In the case of (*E*)-2-hexen-1-al, 5-methyfurfural, phenylacetaldehyde, and vanillin, the matrix effect is similar in white and red wines. In all other analytes, the matrix effect was different depending on the type of wine. On the other hand, one can consider that the matrix effect is similar within each type of wine given the low standard deviation values

Table 3. Ranges of Concentrations ($n = 8$), Limits of Detection (LOD), Intercepts (a), Slopes (b), Regression Coefficients (R^2), and Relative Standard Deviations (% RSD) of Reproducibility and Repeatability

compound	lineal range (μg/L)	LOD (μg/L)	a	b	R^2	% RSD for reproducibility	% RSD for repeatability
2,3-pentadione	2.01−251.7	2.01	0.001	2.45	0.999	4.10	5.46
hexanal	2.01−354.3	2.01	−0.003	28.7	0.999	3.34	4.44
(*E*)-2-hexen-1-al	3.99−153.2	3.99	0.020	61.4	0.999	3.40	4.18
octanal	2.02−364.8	2.02	−0.369	594	0.999	4.02	3.21
acetoin	129.2−20288	129.2	0.003	0.023	0.998	4.10	4.20
(*E*)-2-octenal	3.14−292.9	3.14	0.141	526	0.999	3.81	3.38
furfural	2.01−450.3	2.02	0.018	8.62	0.999	2.90	1.94
decanal	5.01−251.7	5.01	0.191	358	0.999	3.91	4.63
(*E*)-2-nonenal	3.98−351.2	3.98	−0.015	70.7	0.999	1.37	2.62
benzaldehyde	7.96−308.7	7.96	0.027	640	0.999	3.90	3.99
5-methylfurfural	1.82−358.7	1.82	0.023	15.6	0.999	2.90	4.65
(*E*)-2-(*Z*)-6-nonadienal	1.68−151.2	1.68	0.030	175	0.999	4.88	3.16
phenylacetaldehyde	1.68−154.5	1.68	0.033	79.3	0.999	4.60	3.22
acetophenone	1.89−152.8	1.89	−0.032	512	0.999	3.84	2.64
(*E*,*E*)-2,4-decadienal	2.05−148.4	2.05	−0.004	21.0	0.999	3.02	4.34
β-damascenone	0.62−48.8	0.62	−0.007	316	0.999	4.76	4.39
benzophenone	0.88−256.3	0.88	0.019	20.2	0.999	1.85	1.79
vanillin	3.16−149.0	3.16	0.000	1.06	0.999	4.61	5.09

Table 4. Media (%) and RSD (%) of Recoveries

	low level				high level				recovery promedium			
	white wine		red wine		white wine		red wine		white wine		red wine	
compound	media	RSD	media	RSD	media	RSD	media	RSD	media	RSD	media	RSD
2,3-pentadione	43.8	3.4	56.5	2.6	45.2	6.5	55.3	1.9	44.5	8.7	55.9	2.4
hexanal	59.3	2.7	89.0	0.5	61.9	3.2	89.6	1.5	60.6	3.6	89.3	1.1
(*E*)-2-hexen-1-al	31.8	4.0	30.4	4.1	31.2	0.7	29.8	2.8	31.5	2.9	30.1	3.4
octanal	81.8	4.0	98.5	4.4	81.0	2.0	96.9	2.1	81.4	3.0	97.7	3.3
acetoin	146.6	7.5	195.4	3.1	147.8	8.2	188.0	4.9	147.2	11.3	191.7	4.3
(*E*)-2-octenal	42.5	1.8	19.9	7.3	40.4	1.9	19.6	3.4	41.4	3.2	19.8	5.3
furfural	106.6	3.8	79.0	6.0	107.3	2.1	76.1	1.6	106.9	2.9	77.6	4.6
decanal	72.1	7.0	128.4	4.9	72.9	2.9	125.4	3.3	72.5	5.0	126.9	4.1
(*E*)-2-nonenal	44.3	4.5	84.5	4.0	44.6	3.6	83.6	3.2	44.4	7.0	84.1	3.4
benzaldehyde	39.4	7.4	57.3	6.2	39.3	5.5	56.9	2.1	39.4	6.1	57.1	4.3
5-methylfurfural	61.1	4.0	56.6	1.0	59.5	2.7	56.2	2.1	60.3	3.5	56.4	1.6
(*E*)-2-(*Z*)-6-nonadienal	70.8	2.7	30.9	4.7	69.6	3.1	30.7	3.0	70.2	2.9	30.8	3.6
phenylacetaldehyde	31.0	5.6	28.4	4.4	31.4	2.6	30.1	3.2	31.2	4.1	29.3	4.7
acetophenone	79.6	4.5	62.7	2.4	80.6	1.4	62.2	2.0	80.1	3.1	62.5	2.1
(*E*,*E*)-2,4-decadienal	116.0	5.4	104.2	5.3	114.4	3.6	103.2	5.5	115.2	4.3	103.7	5.0
β-damascenone	93.2	4.3	27.6	1.7	90.7	3.1	27.8	2.4	92.0	3.8	27.7	2.0
benzophenone	111.4	6.9	104.4	3.9	111.8	2.6	104.2	2.0	111.6	4.8	104.3	2.9
vanillin	185.8	5.0	187.4	4.1	179.2	2.7	193.7	3.7	182.5	4.2	190.6	4.0

(< 8.2% in white wines and < 7.3% in red wines), such that the average recoveries for each type of wine can be calculated. **Table 4** shows the global mean recovery and relative standard deviation of each analyte for each type of wine obtained by averaging the two doped concentration levels in each type of wine. These recovery values could be used for quantitative purposes. As one can see, there is no definite pattern of behavior: in some cases the recoveries of red wines are higher than those of white wines, in other cases the reverse is observed, while still others are equal.

Quantification in Real Samples of Wines. The developed method was applied to determine the different carbonyl compounds in samples of commercial white and red wines of the Canary Islands. **Table 5** shows the mean value of concentration (micrograms per liter) and standard deviation obtained for the different analytes in 35 white wines and 27 red wines.

The obtained results for the analyzed wines are consistent with those obtained for other wines from different regions of Spain (*42*−*44*).

As one can observe, acetoin is the major component in all wines. However, (*E*)-2-hexen-1-al was not detected in any red wine sample. The analysis of variance indicated that 2,3-pentadione, octanal, acetoin, (*E*)-2-octenal, furfural, 5-methylfurfural, (*E*)-2-(*Z*)-6-nonadienal, phenylacetaldehyde, β-damascenone, and vanillin showed significant differences in mean content between both types of wines, with a higher average content in red wines in all cases, with the exception of (*E*)-2-(*Z*)-6-nonadienal in white wines.

In conclusion, a method for the determination of carbonyl compounds in wines using headspace microextraction combined with high-resolution gas chromatography has been optimized. The DVB/CAR/PDMS fiber performed best during the optimization experiments. Optimized parameters influencing the extraction were as follows: 45 min extraction time, 40 °C extraction temperature, 250 °C desorption temperature, 10 min desorption time, and saturated in sodium chloride. A sulfur dioxide concentration higher than 40 mg/L decreases the peak areas, this problem being resolved using internal standards with a carbonyl group in their structure, in our case 2-nonanone and 2-aminoacetophenone. These same internal standards also corrected the effect of the variation of the alcohol content in the peak areas.

Table 5. Concentration Media and SD (micrograms per liter) of Real Samples of Red and White Wines

	white wines (n = 43)		red wines (n = 27)		
compound	media	SD	media	SD	significant difference ($p < 0.05$)
2,3-pentadione	37.4	55.7	120.0	49.2	yes
hexanal	33.6	81.7	21.4	48.5	–
(*E*)-2-hexen-1-al	d-nq[a]	–	nd[b]	–	–
octanal	33.9	38.7	68.9	86.0	yes
acetoin	4768	2746	7943	6540	yes
(*E*)-2-octenal	13.4	21.2	84.8	53.9	yes
furfural	84.0	38.7	255.3	303.7	yes
decanal	50.4	51.9	47.0	41.5	–
(*E*)-2-nonenal	87.8	38.4	97.2	24.6	–
benzaldehyde	67.4	37.3	51.3	48.0	–
5-methylfurfural	40.2	41.5	66.7	67.1	yes
(*E*)-2-(*Z*)-6-nonadienal	33.3	29.9	17.0	21.6	yes
phenylacetaldehyde	39.8	15.1	51.7	21.7	yes
acetophenone	25.2	22.9	15.5	14.6	–
(*E*,*E*)-2,4-decadienal	27.5	37.2	29.3	40.9	–
β-damascenone	10.1	6.7	17.5	8.6	yes
benzophenone	38.6	34.5	46.2	41.6	–
vanillin	12.8	21.9	29.2	26.4	yes

[a] Detected, not quantified. [b] Not detected.

Tannins, anthocyans, pH, and sugar content did not influence the extraction. Most of the analytes showed a matrix effect, which, in general, is different in white and red wines; thus, a study of recoveries for each type of wine was performed. The developed method has been successfully applied to quantify the analytes in samples of commercial wines.

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