Validation of a Solid-Phase Microextraction Method for Headspace Analysis of Wine Aroma Components

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Abstract: A validated method for identification and quantitation of key aroma components in wine was developed using a divinylbenzene/Carboxen/polydimethylsiloxane solid-phase microextraction fiber combined with gas chromatography/mass spectrometry. Headspace solid-phase microextraction with automated sample handling produced good sensitivity and reproducibility. Reliable quantitation was obtained for components that demonstrated significant headspace depletion and competition for the fiber coating during extraction. Several internal standards were used to target the extraction and chromatographic behavior of each class of analytes in the matrix. Good results were obtained for esters, alcohols, and terpenes. Organic acids were not effectively analyzed using this method. Limits of detection for most target aroma components were in the µg/L range.

Key words: solid-phase microextraction, gas chromatography, headspace analysis, aroma components

The relationship between the chemical composition of wines and sensory evaluation is complex. Several hundred compounds have been identified as important contributors to wine aroma; most are present only in trace amounts (Etiévant 1991, Ebeler 2001). These components comprise several classes of organic compounds, including esters, alcohols, organic acids, ketones, aldehydes, and monoterpene alcohols. Many studies focus on particular classes or groups of components to avoid interference from other components or to increase sensitivity. Mestres et al. (2000, 2002) analyzed high and low volatility sulfur components in wine using flame photometric detection. Both solvent extraction and headspace solid-phase microextraction (HS-SPME) were employed. Vianna and Ebeler (2001) studied ester formation in grape juice fermentations using HS-SPME, while Frivik and Ebeler (2003) assessed the influence of sulfur dioxide on the formation of aldehydes in white wine. Several researchers developed a method to analyze microextracts for a wide range of compounds and then applied it to special situations such as oxidized wines or impact odorants of various varietal and regional wines (Escudero et al. 2004, A. Ferreira et al. 2003, V. Ferreira et al. 2002, 2003, López et al. 2003). Hayasak et al. (1999) analyzed diacetyl in wine using SPME combined with gas chromatography-mass spectrometry (GC-MS). Sala et al. (2000, 2002) used SPME combined with nitrogen-phosphorous detection to quantify 3-alkyl-2-methoxypyrazines in musts and wines in the low ppt range. Vas et al. (1998) developed a method using HS-SPME for rapid screening of a wide range of wine volatiles, but it was only semi-quantitative. Pozo-Bayón et al. (2001) not only analyzed volatiles of several different chemical classes, but also further provided a fully validated method using HS-SPME. In his studies, Guth (1997a,b) identified important wine volatiles based on their odor impact.

Before aroma components can be separated and analyzed, they must be extracted from the wine matrix. Previous studies have identified levels of terpenoids and norisoprenoids (Falqué et al. 2001), esters (Pérez-Coello et al. 1999, Falqué et al. 2001) (S. Dykstra and J. Mike, unpublished data, 2001), and alcohols (Falqué et al. 2001) in representative wines using various extraction and separation methods. Extraction techniques often used for this purpose include static headspace (Villén et al. 1995), purge and trap (Zhang et al. 1994), solid-phase extraction (Arrhenius et al. 1996), and solvent extraction (Vianna and Ebeler 2001). Most of these techniques have several disadvantages, including extensive equipment requirements, significant quantities of expensive and environmentally unfriendly solvents, multiple handling steps that increase error, and a need for concentration of the target analytes to achieve detectable levels.

Despite advantages over other extraction methods, studies using SPME have also demonstrated difficulties. The newer porous solid coatings have shown a nonlinear relationship between the amount of analyte extracted by the fiber and the concentration of that analyte in solution (Górecki et al. 1999). Volatiles exhibit competition for the extraction sites on Carboxen/polydimethylsiloxane (PDMS)

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fibers (Murray 2001), with higher molecular weight compounds displacing those with lower molecular weights. Matich et al. (1996) found while using PDMS-coated fibers that higher molecular weight volatiles equilibrate much more slowly between the sample, headspace, and fiber coating than do lower molecular weight volatiles. In some cases, the headspace may be depleted of one or more high molecular weight components without reaching equilibrium. They concluded that this limits the use of SPME for quantitation of complex systems.

Inconsistent results with standardization methods also appear in SPME studies. For instance, Vaz Freire et al. (2001) concluded that internal standards gave more reliable data than the method of standard additions, while Vianna and Ebeler (2001) found the internal standard method to be unreliable and recommended the use of external standards. One additional study (Ortega et al. 2001) used four internal standards, one for each of the major classes of trace components studied in their research. This approach should help to alleviate the possibility that the internal standard behaves in a significantly different manner than the target components during extraction and separation.

Despite the vast quantity of wine analysis in the literature, there is a strong need to continue to develop efficient, fully validated analytical methods for wine volatile analysis. This research presents a validated SPME method for wine volatile analysis using the 50/30-µm divinylbenzene(DVB)/Carboxen/PDMS fiber. Recent research (Marti et al. 2003) has found that the DVB/Carboxen/PDMS mixed-coating fiber provided efficient extraction of aroma components while also extracting nearly double the number of components extracted with PDMS or polyacrylate fibers. Most studies published thus far have used the 100-µm PDMS fiber. Ferreira and de Pinho (2003) used the 50/30-µm DVB/Carboxen/PDMS fiber and validated this method for several components. The method presented here extends the study to additional compounds of interest.

Materials and Methods

Model solutions and reagents. The standards of different aroma compounds studied were obtained from Sigma-Aldrich (Milwaukee, WI), Fluka (Buchs, Switzerland), Fisher Scientific (Fair Lawn, NJ), and Research Chemicals (Heysham, Lancaster, UK). All reagents used were of the highest purity available and were used as received. Model wine for blank analysis and dilution was prepared in Modulab Type I water (Continental Water Systems, San Antonio, TX) with 12% (v/v) ethanol and 7.0 g/L tartaric acid. The pH was adjusted to 3.3 using 6 *M* NaOH. The standard solution (referred to as MS 150) was prepared from a stock solution in 1:1 water/95% ethanol containing all target analytes at approximately 150% of levels typically found in Chardonnay and Pinot gris wines. This MS 150 solution was then diluted to produce MS 100 containing all target analytes at approximately 100% of typical levels (Table 1).

The internal standard solution was prepared by adding 100 mg 4-methyl-2-pentanol, 100 mg 1,6-heptadien-4-ol, and 4 mg ethyl nonanoate to 125 mL of 95% ethanol. This stock was diluted to a total volume of 250 mL using the model wine described above. For each analysis, 50 µL of the resulting internal standard solution was spiked into each 6-mL sample. Resulting concentrations in the final sample are given in Table 1. The sodium sulfate (Na_2SO_4 •10 H_2O) used for salting out the samples was heated at 210°C for 24 hr to remove the waters of hydration and any low-boiling organic contaminants, then stored in a desiccator.

Wines. Chardonnay and Pinot gris wines were fermented at Youngstown State University using grape must obtained from regional wineries during their October 2001 harvests. Each must was divided into 38-L lots for inoculated (control, C) and spontaneous (S) fermentations. The musts were allowed to settle by gravitation for 24 hr at 4°C. After settling, the musts were racked and portioned into 11-L glass fermentation vessels to provide replicate lots for each grape variety. The control lots were treated with 50 mg/L SO_2 to inhibit the indigenous yeast and then inoculated with commercial freeze-dried yeast (*Prise de Mousse*, EC1118; Lallemand, Montreal, Canada). The spontaneous lots were left untreated and were allowed to ferment via native yeasts present in the must. The vessels were stored at 20°C during fermentation. Once the fermentation process was completed, the wine was racked and the free SO_2 adjusted to 30 mg/L; it was then cold stabilized for 3 weeks at 4°C and bottled.

GC-MS. Ultra-high-purity helium (Praxair, Cleveland, OH) was the carrier gas. Water and oxygen traps were installed on the carrier gas lines. The capillary GC stationary phase was a 30-meter, 250 mm i.d., 0.25-µm film thickness

Table 1 Comparison of analyte peak areas under identical conditions using the 7-µm PDMS fiber (A) and divinylbenzene/Carboxen/PDMS fiber (B).

Analyte	Fiber A	Fiber B	Change with fiber B (%)
Ethyl acetate	59179	121160	+104.7
Ethyl butyrate	147851	294160	+99.0
Ethyl isovalerate	163942	313727	+91.4
Isobutanol	133080	200421	+50.6
Isoamyl acetate	162419	301209	+85.5
3-methyl-1-butanol	268053	409945	+52.9
Ethyl hexanoate	163660	264506	+61.6
Hexyl acetate	166418	244838	+47.1
Ethyl lactate	75940	103511	+36.3
Hexanol	135111	182800	+35.3
Ethyl octanoate	76473	55733	-27.1
Ethyl decanoate	80766	17934	-77.8
Diethyl succinate	138803	16180	-88.3
2-Phenethyl acetate	98502	25275	-74.3
2-Phenethyl alcohol	112174	28981	-74.2

Supelcowax-10 column (Supelco, Bellefonte, PA). All fibers tested, including the manual and autosampler versions of the 50/30-µm DVB/Carboxen/PDMS fiber, were obtained from Supelco and were conditioned according to the manufacturer's instruction before first use (100-µm PDMS 0.5 hr at 250°C; 7-µm PDMS 1 hr at 320°C; 50/30-µm DVB/Carboxen/PDMS 1 hr at 270°C). If not in use for more than 24 hr, then the fibers were cleaned for 20 min at their respective conditioning temperature. The autosampler fibers were custom-fitted by Supelco with a 23-gauge fiber guide to interface with a Merlin Microseal septum (Varian, Palo Alto, CA).

All method validation procedures were conducted on a Varian 3800 GC using a Varian Saturn 2000 ion trap MS/MS detector and STAR version 5.52 chromatography software (Varian, Palo Alto, CA). A starting temperature of 35°C was held constant for 8 min then raised by 3°C per min to 134°C. A final ramp of 20°C per min increased the temperature to 250°C where it was held for 3.2 min. The overall GC run time was 50 min. The carrier gas was ultra-high-purity helium at a constant flow rate of 1.0 mL per min. The injector was held at 260°C. Trap temperatures were as follows: manifold 40°C, transfer line 260°C, and trap 200°C. The mass spectrometer was set in electron ionization mode using a scan time of 0.37 sec/scan and covering a mass-to-charge (*m/z*) range from 25 to 215. The emission current was 10 µA; the maximum ionization time 15,000 µs. Target analytes were identified by comparison of retention times and retention indices with commercial standards and by spectral match with the NIST 98 MS library or the literature. Retention indices for each peak were calculated according to Van den Dool and Kratz (1963). N-alkanes C10 to C25 were added to MS 100, extracted by SPME, and analyzed by GC-MS as described above.

HS-SPME. The GC was fitted with a Combi-Pal Autosampler (CTC Analytics, Zwingen, Switzerland) used in SPME mode throughout validation. The injector was fitted with a Merlin Microseal septum and a 0.75-mm i.d. glass inlet liner. During validation, 10-mL sample vials with magnetic crimp caps and Teflon-lined septa were used. Vials were refrigerated when not in use. Each was used for only one sample, although perhaps for multiple injections, and then discarded. Each sample vial contained 2.1 g Na_2SO_4, 3.0 mL sample (either wine or MS 100), 3.0 mL model wine for dilution, and 50 µL of internal standard solution. The Combi-Pal program was set as follows: vials were heated at 40°C for a 5-min preextraction period with agitation at 500 rpm. The SPME fiber was then inserted into the headspace, where extraction occurred for 30 min with continued heating and agitation. The fiber was subsequently desorbed in the injector for 5 min with a 50:1 split ratio. The autosampler was not equipped with a separate fiber desorption chamber. Therefore, a relatively long desorption time in the injector (5 min) was selected to avoid carryover between runs by ensuring full desorption of all analytes from the fiber. Testing during method development showed this desorption time to be sufficient. The use of a split injection technique together with an 8 min temperature hold at the start of the GC method allowed this approach with no detectable band broadening.

Results and Discussion

HS-SPME parameters. Three common SPME fibers were tested for use in the extraction: 7-µm PDMS, 100-µm PDMS, and 50/30-µm DVB/Carboxen/PDMS (mixed fiber). In agreement with published results (Marti et al. 2003), both PDMS fibers exhibited greater extraction of high molecular weight, nonpolar compounds than the mixed fiber. However, the mixed fiber exhibited significantly greater extraction of both lower molecular weight and more polar compounds (Table 1). The gain in extractability for the polar analytes exceeded the loss of extractability for the nonpolar analytes, and the DVB/Carboxen/PDMS was chosen as the preferred fiber. Ferreira and de Pinho (2003) evaluated seven types of commercially available fibers, including the three tested in these experiments. They also found the 50/30-µm DVB/Carboxen/PDMS fiber to be the most selective for the major components in their study.

Virtually all published methods for the analysis of aroma volatiles in wine use salting out to increase the levels of analytes in the headspace before extraction. A variety of inorganic salts can be used; sodium sulfate was selected in this study because of its high solubility in aqueous solutions and the production of triple-molar amounts of ions upon dissociation. These factors combine to yield high ion loads in the wine, enhancing the effects of salting out. A saturation level of 2.1 g Na_2SO_4 per 6.0 mL aqueous sample was found to be optimal.

Extraction studies were conducted to determine the optimum time to hold the fiber in the headspace of the sample. For example, if the fiber is held in the headspace too long, competition for sites on the fiber could cause inaccuracies in the relative amounts of analytes present. However, an abbreviated sampling time might lead to inconsistencies in concentration if equilibrium is not attained between the aqueous sample, headspace, and fiber. The samples were extracted for predetermined exposure times of 5, 10, 15, 20, 30, or 45 min. Each vial was extracted three times to observe possible differences due to depletion of analytes. The average peak areas of representative analytes were plotted against extraction time to determine the time required for equilibration of each analyte between the headspace and the fiber. Thirty minutes was determined to be the optimum extraction time.

Target analytes and internal standards. During the development process some analytes present in the initial target list were not detected in the available samples of Chardonnay or Pinot gris wines using the SPME fiber and chromatographic conditions described above; these components (including γ-butyrolactone, furaneol, diacetyl, guaiacol, and acetaldehyde) were dropped from the validation. Additionally, the organic acids (isobutyric, butyric,

isovaleric, hexanoic, octanoic, and decanoic acids) exhibited poor extraction and chromatographic behavior, as well as levels in wine that were low enough to justify exclusion from the validation.

The initial stages of method development used several internal standards covering a range of molecular weights and polarities in order to more closely match the varying interactions of the target analytes during extraction and analysis. Compounds were selected to mimic the behavior of the esters (ethyl 2-hydroxyvalerate), organic acids (valeric acid), alcohols (4-methyl-2-pentanol, 2-butanol, and 2-octanol), ketones (acetone and 4-hydroxy-4-methyl-2-pentanone), and monoterpene alcohols (1,6-heptadien-4-ol and 9-decen-1-ol). These potential internal standards were tested using the extraction and separation conditions already established, and the literature was searched to verify that none of these components had been identified as a natural component of wine. Several of these compounds were subsequently removed from the study or replaced because of overlap with other wine components or inappropriate chromatographic behavior. The resulting internal standards used for the remainder of the validation were ethyl nonanoate (esters), 4-methyl-2-pentanol (alcohols), and 1,6-heptadien-4-ol (monoterpene alcohols).

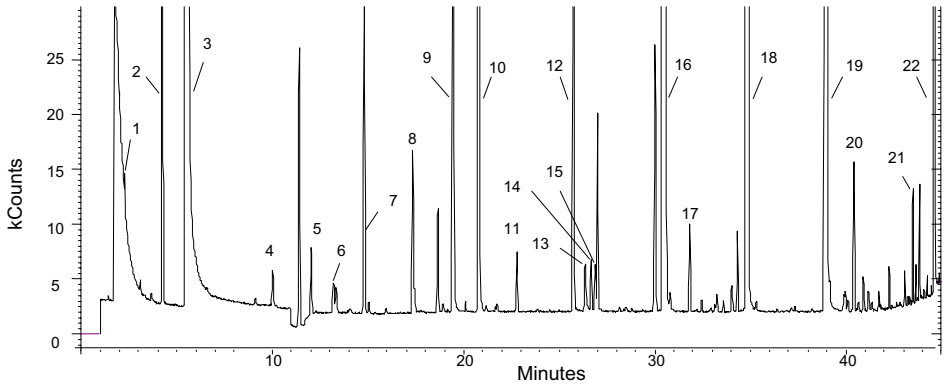

Figure 1 Typical chromatogram from a Pinot gris wine spiked with standards. Peak identification: (1) acetaldehyde, (2) ethyl acetate, (3) ethanol, (4) ethyl butyrate, (5) ethyl isovalerate, (6) isobutanol, (7) isoamyl acetate, (8) 4-methyl-2-pentanol, (9) 3-methyl-1-butanol, (10) ethyl hexanoate, (11) hexyl acetate, (12) 1,6-heptadien-4-ol, (13) ethyl lactate, (14) hexanol, (15) 4-hydroxy-4-methyl-2-pentanone, (16) ethyl octanoate, (17) linalool, (18) ethyl nonanoate, (19) ethyl decanoate, (20) diethyl succinate, (21) 2-phenethyl acetate, and (22) 2-phenethyl alcohol.

GC-MS parameters. It was initially assumed that a splitless injection procedure, commonly used for trace analysis, would produce optimal sensitivity and detection limits. However, the resulting chromatogram showed unacceptable interferences and significant band broadening for a number of peaks. The Saturn 2000 mass spectrometer used in this study ionizes the column effluent in the trap rather than prior to it, as in other instruments. That can result in very high ion concentrations in the trap, causing chemical ionization even at µg/L concentration levels of the analytes.

The first adjustment made to compensate for this difficulty was a 1:1 dilution of all samples with model wine before analysis. Although the results improved, additional alterations to the method proved necessary. Therefore, various split ratios (20:1, 50:1) were tested for both standard solutions and wine samples, together with differing desorption times. The final parameters (a 50:1 split ratio with a 5-min desorption time) were selected for the production of sharp, well-resolved peaks with good validation results. A typical chromatogram is shown in Figure 1. Retention indices of standards and target wine components were the same (Table 2).

Headspace depletion. To determine the possible headspace depletion of specific analytes during the extraction,

Table 2 Aroma standards in MS 100 model solution. Retention indices (RI) were determined on a Supelcowax-10 column. All threshold values are from Guth (1997b) except as indicated.

Analyte	Concn (mg/L)	RI	Threshold (mg/L)	OAV
Ethyl acetate	120	—[a]	7.5	16
Ethyl butyrate	0.60	1034	0.020	30
Isobutanol	13.7	1100	40	0.34
Isoamyl acetate	1.35	1118	0.30	45
3-Methyl-1-butanol	132	1206	30	4.4
Ethyl hexanoate	1.20	1235	0.005	240
Hexyl acetate	0.08	1275	1.5[b]	0.05
Ethyl lactate	300	1347	157.81[b]	1.9
Hexanol	2.00	1355	8	0.25
cis-3-Hexen-1-ol	5.00	1384	0.400	12.5
Ethyl octanoate	1.50	1436	0.002	750
Linalool oxide	6.00	1466	3–5[c]	1.2–2.0
Linalool	0.15	1550	0.015	100
Ethyl decanoate	0.04	1639	0.20[d]	0.20
Diethyl succinate	2.00	1677		
α-Terpineol	0.40	1693	0.330[d]	1.2
β-Citronellol	0.20	1767	0.100	2
Nerol	0.30	1800	0.400	0.75
2-Phenethyl acetate	0.25	1811	0.250	1
Geraniol	0.30	1849	0.030	10
2-Phenethyl alcohol	50	1909	10	5
Internal standards[e]				
4-Methyl-2-pentanol	3.3	1171		
1,6-Heptadien-4-ol	3.3	1330		
Ethyl nonanoate	0.13	1537		

[a]Did not elute within the retention time range of the C10–C20 alkanes.
[b]Etievant (1991).
[c]Ribéreau-Gayon et al. (1975).
[d]Ferreira et al. (2000).
[e]Internal standard components included in separate stock solution; concentration given is the final concentration when spiked into samples.

five vials were prepared using the MS 100 standard solution. The first vial was sampled five times using the extraction time and program described above, the second vial was sampled in triplicate, and the remaining three vials were sampled once each. Peak areas for the multiple runs were averaged, and relative standard deviations (RSD) were calculated for each analyte (Table 3). The first extraction from each of the five vials was also analyzed and RSD values calculated.

A steady pattern of decline in analyte peak area was evident for the higher molecular weight esters (ethyl hexanoate, ethyl octanoate, ethyl decanoate, and hexyl acetate) during multiple extractions from a single vial. Decreases in peak area of up to 82% were observed from the first to the third extraction (Figure 2). Significantly lower RSD values for these components were obtained from averaging single extractions from five separate vials (Table 3). Overall repeatability was determined to be sufficient to allow quantitation of the high molecular weight esters from the first extraction of each sample. For all other compounds, the average of triplicate extractions from a single vial was used to quantify throughout the study.

In agreement with published results for a similar fiber coating (Murray 2001), higher molecular weight compounds appeared to be preferentially extracted by the fiber in the earlier runs. As headspace depletion of these components became significant, a greater proportion of the fiber coating was available for other analytes. Components such as diethyl succinate and linalool oxide exhibited a significant increase in run-to-run peak area during the replicate extractions, providing evidence for potential competition on the coating of the fiber (Figure 2). In contrast to the conclusions of previous studies (Matich et al. 1996), the method presented here provides good quantitation of the highly complex wine matrix even when headspace depletion and competition are observed during extraction.

Specificity. This combined measure of several factors indicates how effectively each analyte was separated from other components. The analyses were conducted using Pinot gris wine. Results are presented in Table 4. The peak with resolution less than 1.0 (4-methyl-2-pentanol), although not fully resolved, was still quantifiable using the mass spectrometric data.

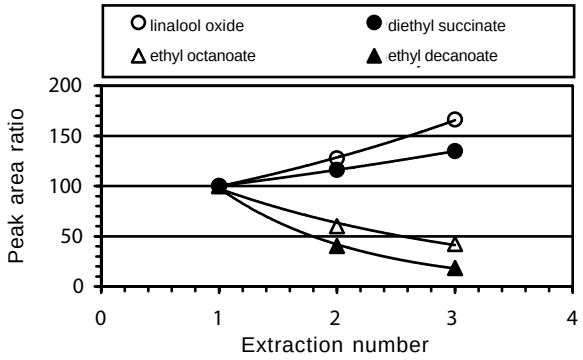

Figure 2 Triplicate run peak area ratios, calculated as a percentage of the first extraction result, for selected analytes.

Table 3 Compound headspace depletion comparison in MS 100 model solution (RSD = relative standard deviation). (A) Five extractions and (B) three extractions from a single vial. (C) One initial extraction from five vials.

Analyte	RSD (A)	RSD (B)	RSD (C)
Acetaldehyde	14.8	27.9	12.5
Ethyl acetate	9.0	3.8	4.9
Ethyl butyrate	16.8	2.2	10.2
Isobutanol	12.4	26.9	12.3
Isoamyl acetate	8.5	5.8	8.0
4-Methyl-2-pentanol	14.2	15.4	14.7
3-Methyl-1-butanol	6.5	28.8	15.8
Ethyl hexanoate[a]	24.7	17.5	2.9
Hexyl acetate[a]	20.2	19.8	2.4
1,6-Heptadien-4-ol	13.1	8.7	8.3
Hexanol	27.1	4.7	28.9
4-Hydroxy-4-methyl-2-pentanone	18.7	3.7	7.4
Ethyl octanoate[a]	46.9	23.8	2.6
Linalool oxide	24.2	10.0	9.2
Ethyl decanoate[a]	114.8	80.1	4.0
Diethyl succinate	14.4	12.7	2.9
2-Phenethyl acetate	4.2	4.1	5.0
2-Phenethyl alcohol	14.3	12.0	5.9

[a]Single extraction used to quantitate these components throughout the study due to headspace depletion.

Table 4 Specificity parameters for key analytes in Pinot gris wine: resolution (R_s), number of theoretical plates or column efficiency (N or N^*), peak symmetry (A_s), and capacity factor (k').

Analyte	R_s	N or N^* (x 10^4)	A_s	k'
Ethyl acetate	1.020	N^* = 5.93	0.636	1.43
Ethyl butyrate	1.157	N = 9.200	1.000	4.635
Isobutanol	11.31	N = 15.46	1.030	6.475
Isoamyl acetate	1.773	N = 19.50	0.9300	7.330
4-Methyl-2-pentanol	0.533	N^* = 37.6	0.863	8.83
3-Methyl-1-butanol	5.077	N^* = 37.68	0.8560	10.03
Ethyl hexanoate	1.024	N^* = 63.34	0.8160	10.79
Hexyl acetate	5.117	N^* = 78.93	1.185	11.95
1,6-Heptadien-4-ol	4.918	N^* = 104.9	0.8710	13.68
Ethyl lactate	2.874	N = 56.37	0.9190	14.03
Hexanol	1.865	N = 99.13	1.038	14.23
4-Hydroxy-4-methyl-2-pentanone	1.895	N^* = 94.47	0.8510	14.35
Ethyl octanoate	3.912	N^* = 128.9	0.6310	16.37
Ethyl nonanoate	1.012	N^* = 122.4	0.6260	18.88
Ethyl decanoate	1.033	N^* = 240.4	0.7400	21.22
Diethyl succinate	4.710	N^* = 288.3	0.8890	22.10
2-Phenethyl acetate	1.373	N^* = 1247	0.6490	23.88
2-Phenethyl alcohol	3.088	N^* = 1915	0.7000	24.51

Limits of quantitation (LOQ) and detection (LOD). These parameters were calculated as the minimum concentration that generated a peak signal at least 10 times higher (LOQ) or three times higher (LOD) than the signal from adjacent noise. This ensured quantitation with no more than 10% error because of noise and the ability to differentiate a peak from random noise. Determination was made by calculating the average signal-to-noise ratio (S/N) from triplicate runs of a series of standard dilutions of target wine analytes and internal standards; results are shown in Table 5. Ethyl isobutyrate was not found in wines at levels sufficient for detection and was subsequently dropped from the analysis. Acetaldehyde had high limits of detection and quantitation because of its presence as a small tangent on the side of a significant air peak resulting from fiber insertion into the injector; when the validated range did not include the levels subsequently observed in wines, it was also dropped from the analysis.

Linearity. Average peak area was plotted versus concentration for each analyte in the standard solution and the resulting plots were analyzed for linearity (Table 5). Isobutanol was the only component that was observed to have a linear range that did not extend to 0 mg/L; low concentrations of this analyte exhibited a nearly zero slope, indicating levels below the detection limit.

Sensitivity. If a method is suitably sensitive for a particular analyte, then increases in the concentration of that analyte should produce a measurable increase in response. The sensitivity of the method for each analyte in the standard solutions and in the wine matrix is given by the slope of the associated curve. Standard additions were made in the Pinot gris wine matrix (Table 5). Curves with similar slopes are expected if there is no matrix effect; the Pinot gris matrix exerted a dampening effect on method sensitivity for most analytes.

Table 5 Validation parameters: limit of detection (LOD), limit of quantitation (LOQ), sensitivity in standard solution (S_S), sensitivity in spiked Pinot gris (S_{PG}).

Analyte	LOD (mg/L)	LOQ (mg/L)	Standard solution calibration line	R^2	Linear range (mg/L)	S_S (counts/mg/L)	S_{PG} (counts/mg/L)	Validation range (mg/L)
Acetaldehyde	17.70	53.10	y = 118.5x - 85.13	0.9875	nd – 106.1	118.0	78.00	53.10–106.1
Ethyl acetate	0.3347	1.521	y = 3249x + 2045	0.9941	nd – 83.67	3249	2655	1.521–83.67
Ethyl isobutyrate	0.1308	>0.2616	NA[b]	NA[b]	NA[b]	NA[b]	NA[b]	NA[b]
Ethyl butyrate	0.01423	0.05693	y = 58370x + 995.2	0.9863	nd – 0.5123	58370	82040	0.05693–0.5123
Ethyl isovalerate	0.00056	0.0041	y = 1.3E6x + 336.6	0.9921	nd – 0.037	1.3E6	1.1E6	0.0041–0.037
Isobutanol	0.311345	2.28320	y = 3394.70x - 85.6000	0.9645	3.42480 – 10.2744	3395.00	2940.00	2.28320–10.2744
Isoamyl acetate	0.0067073	0.030488	y = 189360x + 1729.5	0.9904	nd – 0.83842	189400	154020	0.030488–0.83842
4-Methyl-2-pentanol[a]	0.159288	0.289615	y = 13693.0x + 664.380	0.9883	nd – 9.55730	13690.0	9493.00	0.289615–9.55730
3-Methyl-1-butanol	0.1658	0.6630	y = 8138x + 1407	0.9818	nd – 132.6	8138	7251	0.6630–132.6
Ethyl hexanoate	0.0030505	0.0061010	y = 594420x + 5950.7	0.9692	nd – 1.2202	594400	420800	0.0061010–1.2202
Hexyl acetate	0.001762	0.009690	y = 457400 x + 48.02	0.9830	nd – 0.02908	457400	543500	0.009690–0.02908
1,6-Heptadien-4-ol[a]	0.0323048	0.161524	y = 26789.0x + 4459.10	0.9822	nd – 9.69145	26790.0	8578.00	0.161524–9.69145
Ethyl lactate	1.735	9.542	y = 825.4x - 1798	0.9686	nd – 57.25	825.0	427.0	9.542–57.25
Hexanol	0.033515	0.18433	y = 33452x + 876.29	0.9480	nd – 1.8433	33450.	26970.	0.18433–1.8433
4-Hydroxy-4-methyl-2-pentanone[a]	2.814	16.88	y = 829.2x - 1376	0.9754	nd – 50.65	829.0	731.0	16.88–50.65
Ethyl octanoate	<0.0008546	0.0008546	y = 1505000x + 5517	0.9960	nd – 2.564	1505000	530500	0.0008546–2.564
Linalool oxide	0.054177	0.098546	y = 19408x + 1937.4	0.9786	nd – 3.2506	19410.	16680.	0.098546–3.2506
Ethyl nonanoate[a]	0.000933856	0.00466928	y = 1128640x + 43303.10	0.9910	nd – 2.80157	1129000	-345010.	0.00466928–2.80157
Ethyl decanoate	0.00621406	0.186422	y = 752760.x - 6041.90	0.9840	nd – 3.72843	752800.	54350.0	0.186422–3.72843
Diethyl succinate	0.014924	0.067836	y = 55111x + 893.50	0.9841	nd – 1.8655	55110.	41040.	0.067836–1.8655
2-Phenethyl acetate	0.003612	0.01987	y = 2522x - 459.8	0.9914	nd – 0.1589	252200	186400	0.01987–0.1589
2-Phenethyl alcohol	0.008911	0.2228	y = 10880x - 1527	0.9984	nd – 53.47	10880	8827	0.2228–53.47

[a]Internal standard.
[b]Analyte removed from the analysis.

Recovery. This measure of the accuracy of a method is often used when a standard of exactly known concentrations and identical matrix to the sample is not available for comparison. Three vials were extracted in triplicate: vial 1 (MS 100), vial 2 (1:1 mixture of MS 100 and wine with internal standards [IS] added), and vial 3 (wine with IS). The expected peak area (PA) of the mixed vial (vial 2) for each analyte and IS was calculated according to the following equation:

Expected PA vial 2 = (½ PA vial 1) + (½ PA vial 3)

The percent recovery (%R) calculation for each analyte and IS used the following:

%R = [PA vial 2 / expected PA vial 2] x 100

Corrected recoveries were also calculated using peak area ratios (PA analyte/PA relevant IS) in place of peak areas. The internal standards used for correction and the recovery results are shown in Table 6. Components that had resolution difficulties showed poor recoveries. Acetaldehyde (a small tangent peak) along with 3-methyl-1-butanol and linalool oxide (which sometimes coeluted with fiber-bleed peaks) showed problematic run-to-run repeatability, which likely contributed to poor recovery results for these analytes. Additionally, the ketone used as the internal standard for acetaldehyde was a poor match because of its significantly higher molecular weight and hydroxyl group substituent. This standard was later dropped from the analysis.

Precision. Two primary measures of precision are injection repeatability and intra-assay repeatability. Injection repeatability is a measure of the random variability that occurs when the same sample is injected multiple times in an identical manner, measured by calculating the RSD of peak areas for selected components from five injections of the same vial. Only those components that did not demonstrate headspace depletion as described above were used for this validation. Intra-assay repeatability is the variation between identical samples prepared and analyzed separately using the same method and was determined from the RSD of the peak areas of single extractions from five separate but identically prepared vials. Headspace depletion had no impact on this parameter. Results are shown in Table 7. All components were quantified with acceptable precision using this method. The alcohols exhibited a few higher RSD values, probably related to competition with high molecular weight esters for sites on the fiber as discussed above. Additionally, the RSD of the retention times for both injection and intra-assay repeatability were calculated and found to be quite good for all components (Table 7). That could be useful in future work if narrow time windows for selected-ion monitoring MS are desired in order to improve the detection of some components.

Validation range. This parameter was determined by synthesizing the data from the linearity, precision, and limit of quantitation and detection sections. This method is valid across the ranges presented in Table 5.

Stability. Chardonnay wine spiked with internal standards was used to prepare three sets of 15 vials each.

Table 6 Percent recoveries for selected analytes.

Analyte	%R Pinot gris	Corrected %R Pinot gris	%R Chardonnay	Corrected %R Chardonnay
Acetaldehyde	176.6	46.9	131.5	142.5
Ethyl acetate	103.6	101.7	101.4	88.0
Ethyl butyrate	110.4	126.6	92.3	90.9
Ethyl isovalerate	77.4	100.7	45.8	76.9
Isobutanol	135.6	96.3	127.1	66.7
Isoamyl acetate	106.4	121.8	88.8	100.1
4-Methyl-2-pentanol[(1)a]	107.7	100.0	123.2	100.0
3-Methyl-1-butanol	123.7	63.1	113.1	55.2
Ethyl hexanoate	107.5	123.7	NA[b]	NA[b]
Hexyl acetate	109.4	112.9	76.1	91.4
1,6-Heptadien-4-ol[(2)a]	113.8	100.0	113.1	100.0
Ethyl lactate	112.0	113.6	113.5	153.4
Hexanol	96.9	63.3	100.0	30.9
4-Hydroxy-4-methyl-2-pentanone[(3)a]	124.1	100.0	145.8	100.0
Ethyl octanoate	77.1	93.2	93.6	100.0
Linalool oxide	129.3	33.9	81.9	43.9
Ethyl nonanoate[(4)a]	89.2	100.0	93.6	100.0
Ethyl decanoate	80.8	170.1	88.7	93.0
Diethyl succinate	125.8	147.1	115.8	110.5
2-Phenethyl acetate	112.7	114.3	100.2	106.7
2-Phenethyl alcohol	89.6	47.6	114.6	35.4

[a]Internal standards: [(1)] alcohols; [(2)] linalool oxide, [(3)] aldehydes, [(4)] esters.
[b]Error occurred during analysis.

Table 7 Precision results for selected analytes.

	Injection repeatability		Intra-assay repeatability	
Analyte	Peak area RSD	Retention time RSD	Peak area RSD	Retention time RSD
Ethyl acetate	9.0	0.67	4.9	0.78
Isoamyl acetate	8.5	0.64	8.0	0.74
3-Methyl-1-butanol	6.5	0.40	15.8	0.57
2-Phenethyl acetate	4.2	0.03	5.0	0.07
2-Phenethyl alcohol	14.3	0.02	5.9	0.05

One vial from each set was analyzed immediately to provide baseline (day 0) data. Remaining vials were stored at different temperatures: room temperature (25°C), refrigerated (4°C), and frozen (-16°C). On selected days during storage, a fresh vial was prepared and analyzed together with one vial from each storage set. Representative analytes (3-methyl-1-butanol, 1,6-heptadien-4-ol, ethyl octanoate, and diethyl succinate) were selected for the determination of stability. Analyte peak areas for the stored vials were divided by the analyte peak areas from the fresh vial to produce daily peak area ratios for each analyte. A ratio of 1.0 indicates no difference between the peak areas of the fresh and the stored vials. The peak area ratios were plotted against time of storage for each of the three storage temperatures and analyzed for a pattern of stability. Data from the first five days are questionable because a fiber was later determined to be flawed. All four components were stable for at least the first 11 days, with ethyl octanoate consistently above a ratio of 1.0 and 1,6-heptadien-4-ol consistently below that ratio.

Conclusions

This research presents a validated SPME-GC method for the analysis of essential trace components in Pinot gris and Chardonnay wines using the DVB/Carboxen/PDMS mixed-coating fiber. The most notable departures from previously described methods for trace aroma analysis involved the selection of split injection and a 1:1 dilution of samples to avoid chemical ionization in the trap of the mass spectrometer. Overall, this validated method provides good reproducibility, limits of detection, and sensitivity for a number of key components in wine aroma. For most components the concentration range covers the concentration range of wine volatiles present in white wines, rosé wine, and red wines.

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