Application Note

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Analysis of Free Volatile Phenols in Smoke-Impacted Wines by SPME

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Abstract

Ever since the 2003 wildfires in Australia and British Columbia, smoke impact has been a global concern for wine production.[1] With the increase in wildfires over various regions around the globe, many growers and wineries continue to worry about smoke impact in grapes and their wine. Agilent has developed a solid phase microextraction (SPME) gas chromatography/mass spectrometry (GC/MS) method to analyze the free-form volatile phenols associated with smoke impact. The Agilent SPME-GC/MS/MS method for the analysis of free-form volatile phenols associated with smoke impact allows for confident identification and reliable quantitation.

Introduction

Research has shown that smoke compounds can be absorbed by vines and grapes causing off-flavors in wines. While there is strong evidence that these compounds are mostly present in grapes and juice as nonvolatile forms, analysis of their free fraction has been used as a tool for screening grapes and assessing impacts in wines.[2] In the wine making process, the growth and maturation of the grape is arguably the most important step. During the period of veraison, acid concentration decreases, and sugar concentration increases while aromatic and flavor compounds start to develop. There are many external factors, weather conditions being the most influential, that determine when grapes have matured and are ready for harvest. Other environmental conditions, unrelated to temperature, such as smoke from nearby fires, can have a large and negative impact on the sensory quality of the wine.[3]

Guaiacol and 4-methylguaiacol have been identified as the primary volatile aromatics that contribute to the undesirable smoke impact characteristic. While aging wine in oak barrels can also contribute to the concentration of guaiacol and 4-methylguaiacol, the ratio of these two compounds will differ. Smoke-impacted berries contain almost four times as much guaiacol as 4-methylguaiacol.[2] The aroma contributed by oak barrels will be perceived as smoke and char. In contrast, when the two compounds are present due to smoke impact, it will be more reminiscent of campfires and ashtrays, which is not desirable in wine.

Detection limits for the analysis of smoke impact compounds must be sensitive enough to detect below 1 ppb, which is why selected ion monitoring (SIM) or multiple reaction monitoring (MRM) are commonly used in GC/MS analyses. Direct analysis of wine can be challenging because of the sugars, organic acids, and other aromatic compounds with higher retentions. To simplify the extraction and analysis of these volatiles, SPME has become the extraction method of choice. Its popularity for use stems from its operational simplicity, suitability for automation, reduced use of organic solvents, and direct thermal desorption into a gas chromatograph.

Experimental

Target volatiles

The main volatile phenols in smoke, guaiacol and 4-methylguaiacol, are useful markers of smoke impact in wines. Their respective concentrations correlate with the degree of perceived smoke impact, particularly in wines not exposed to toasted oak. However, they are not the only two compounds that are found and analyzed in smoke-affected wines, Table 1 lists the target free form volatile phenols that were analyzed in this experiment.

Table 1. Target free form volatile phenols.

CAS Number	Compound
74495-69-5	Guaiacol-d3
90-05-1	Guaiacol
93-51-6	4-Methylguaiacol
95-48-7	o-Cresol
13127-88-3	Phenol-d6
108-95-2	Phenol
95-87-4	2,5-Xylenol
2785-89-9	4-Ethylguaiacol
90-00-6	2-Ethylphenol
108-68-9	3,5-Xylenol
106-44-5	p-Cresol
108-39-4	m-Cresol
123-07-9	4-Ethylphenol
91-10-1	2,6-Dimethoxyphenol

Method

Sample preparation:

- 20 mL headspace vial and cap (part numbers 5188-6537 and 5188-2759)
- 10 mL sample with 4 g NaCl (Figure 1)
  - Addition of NaCl to saturation increases response for target compounds in smoke-affected grapes and wine by an average of 95%[4]

Water **Wine**

Figure 1. 20 mL amber headspace vials with water and wine samples.

- Samples spiked with calibrators and/or internal standards (ISTDs)
  - ISTDs spiked in at 10 ppb
- Agilent SPME Arrow DVB/carbon WR/PDMS, 1.10 mm, 120 µm (part number 5191-5861)
  - DVB/carbon WR/PDMS SPME phase was chosen for its selective extraction of odor and flavor compounds
  - SPME Arrow was used because of its significant benefit in extraction efficiency due to its larger sorption phase volume, compared to a traditional SPME fiber[5]

An Agilent PAL3 autosampler with robotic tool change (RTC) was installed on an Agilent 8890 GC system with an Agilent 7000D triple quadrupole GC/MS. The SPME headspace parameters, GC method settings, and MS conditions are listed in Tables 2, 3, and 4, respectively. Table 5 provides the MRM transitions used for GC/MS/MS analysis.

Table 2. SPME headspace parameters.

Parameter	Setting
Predesorption Time	3 min
Predesorption Temperature	250 °C
Incubation Time	5 min
Heatex Stirrer Speed	1,000 rpm
Heatex Stirrer Temperature	40 °C
Sample Extract Time	10 min
Sample Desorption Time	3 min

Table 3. Agilent 8890 GC settings.

Parameter	Setting
Inlet Liner	Agilent Ultra Inert inlet liner, splitless, straight, 0.75 mm id, recommended for SPME injections (p/n 5190-4048)
Injection Mode, Temperature	Splitless, 250 °C
Control Mode	Constant flow (1.2 mL/min)
Column	Agilent J&W DB-HeavyWAX GC column, 30 m, 0.32 mm, 0.25 µm (p/n 123-7132)
Oven Program	120 °C (hold 1 min); 10 °C/min to 250 °C (hold 0 min); 60 °C/min to 280 °C (hold 0 min)

Table 4. Agilent 7000D triple quadrupole GC/MS conditions.

Parameter	Setting
Transfer Line	280 °C
Acquisition Mode	dMRM
Solvent Delay	3.0 min
Tune File	Atune.eiex
Gain	10
MS Source Temperature	280 °C
MS Quadrupole Temperature	150 °C

Table 5. MRM transitions for free form volatile phenols.

CAS Number	Compound	Precursor Ion (*m/z*)	Product Ion (*m/z*)	CE (V)
74495-69-5	Guaiacol-d3	124.1	109	15
		124.1	81	15
90-05-1	Guaiacol	127	109	15
		126.9	109	15
93-51-6	4-Methylguaiacol	138.1	95	15
		138	123	15
95-48-7	*o*-Cresol	108.1	107.1	15
		107.1	77	15
13127-88-3	Phenol-d6	99.1	71	10
		71	69	10
108-95-2	Phenol	94	66	10
		66	65	10
95-87-4	2,5-Xylenol	122	107	15
		122	94	15
2785-89-9	4-Ethylguaiacol	152	137	15
		137.1	122	15
90-00-6	2-Ethylphenol	122.1	107.1	15
		107.1	77	15
108-68-9	3,5-Xylenol	121.1	107.1	15
		121.1	77	15
106-44-5	*p*-Cresol	108.1	107.1	15
		107.1	77	15
108-39-4	*m*-Cresol	108.1	107.1	15
		107.1	77	15
123-07-9	4-Ethylphenol	122.1	107	15
		108.1	78	15
91-10-1	2,6-Dimethoxyphenol	154	139	15
		139.1	83	15

Results and discussion

Calibration

Blanks are important for quality control and robust quantitative analytical methods. In this experiment, Milli-Q (18.2 Ω) water was used as a blank to simulate a clean matrix without any interferences. However, since wine includes many components that can affect the measurement of the target analytes, white wine was used as a matrix blank.

Table 6 provides the calibration ranges and linearity values for the target free form volatiles when calibrated in Milli-Q water. Figure 2 shows guaiacol and 4-methylguaiacol Milli-Q water calibration curves together.

To account for matrix effects in quantitating guaiacol and 4-methylguaiacol, a bag-in-a-box white wine was chosen. The reasons this matrix was chosen were:

- The skins, where smoke impact compounds reside, are separated from the juice before the fermentation process.
- It is an unspecified blend, which represents a broader matrix.
- The packaging removes the exposure of oak and cork from the wine.

Table 6. Agilent 7000D triple quadrupole GC/MS calibration range and R^2 in Milli-Q water.

Compound	Calibration Range (ppb)	R^2
Guaiacol	0.2 to 50.3	0.999
4-Methylguaiacol	0.1 to 25	0.999
o-Cresol	0.2 to 50	0.996
Phenol	0.5 to 125.5	0.997
2,5-Xylenol	0.1 to 25	0.998
4-Ethylguaiacol	0.1 to 25	0.998
2-Ethylphenol	0.03 to 7.5	0.995
3,5-Xylenol	0.1 to 5	0.998
p-Cresol	0.1 to 25	0.997
m-Cresol	0.1 to 25	0.998
4-Ethylphenol	0.1 to 25	0.998
2,6-Dimethoxyphenol	0.1 to 25	0.998*

* Type = quadratic, origin = force; weight = 1/x.

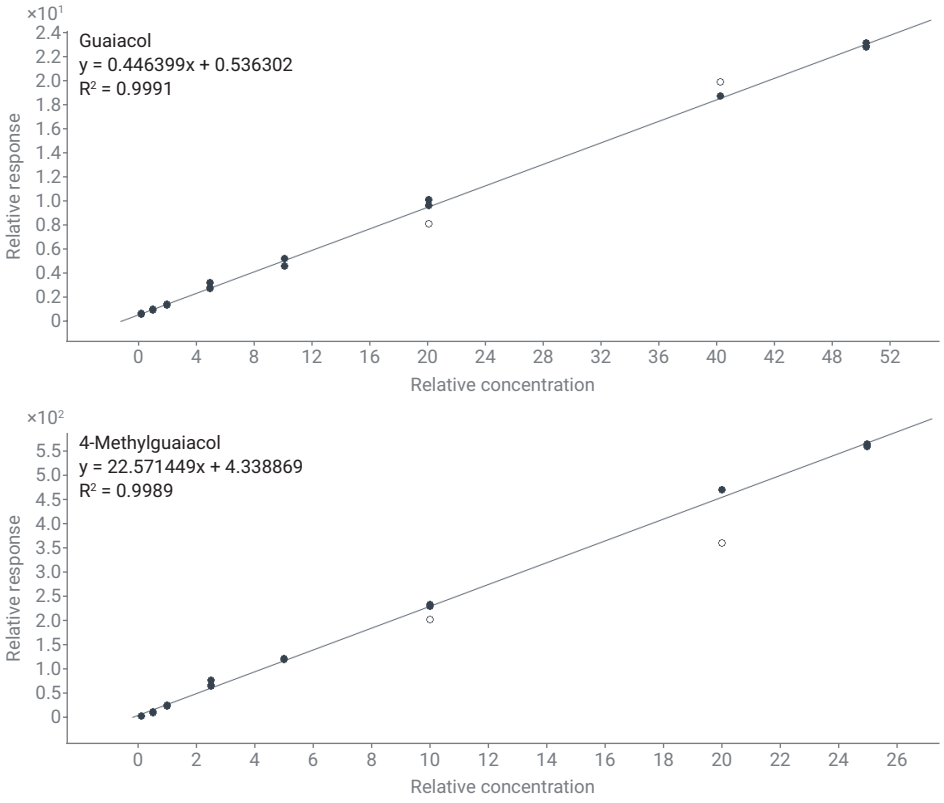

Figure 2. Calibration curves for guaiacol and 4-methylguaiacol in Milli-Q water.

Table 7 provides the calibration ranges and linearity values for the target free form volatiles when calibrated in the bag-in-a-box white wine. Figure 3 shows guaiacol and 4-methylguaiacol white wine calibration curves together.

Table 7. Agilent 7000D triple quadrupole GC/MS calibration range and R^2 in white wine.

Compound	Calibration Range (ppb)	R^2
Guaiacol	0.2 to 50.3	0.993
4-Methylguaiacol	0.1 to 25	0.996
o-Cresol	0.2 to 50	0.996
Phenol	0.5 to 125.5	0.997
2,5-Xylenol	0.1 to 25	0.996
4-Ethylguaiacol	0.1 to 25	0.996
2-Ethylphenol	0.03 to 7.5	0.995
3,5-Xylenol	0.1 to 5	0.998
p-Cresol	0.1 to 25	0.995
m-Cresol	0.1 to 25	0.995
4-Ethylphenol	0.1 to 25	0.996
2,6-Dimethoxyphenol	0.1 to 25	0.995

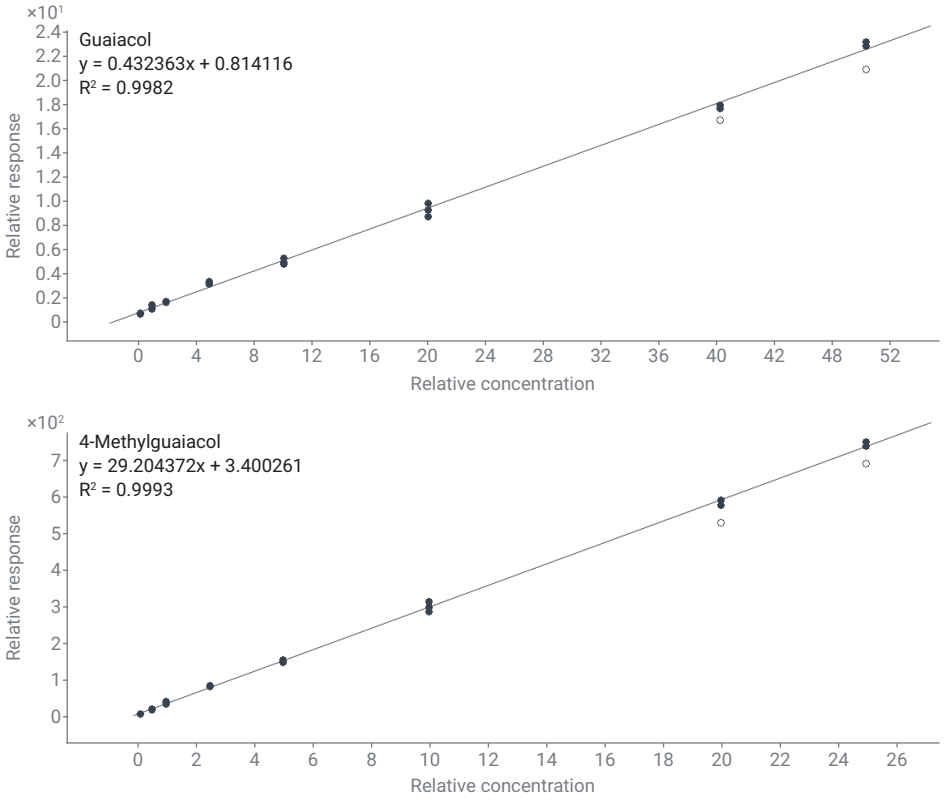

Figure 3. Calibration curves for guaiacol and 4-methylguaiacol in white wine.

Quantitation of smoke impact markers

In grapes not exposed to smoke, levels of 0.1 to 0.3 ppb for both guaiacol and 4-methylguaiacol can be observed. Guaiacol levels above 1 ppb could suggest exposure to smoke, and levels of guaiacol in smoke-exposed grapes have been as high as 55 ppb. On average, a ratio of 3.7/1 guaiacol/4-methylguaiacol is observed in undesirable smoke-impacted grapes and wine.[2]

Guaiacol and 4-methylguaiacol levels in all wine samples and the white wine blank, signals were quantitated based on the Milli-Q water calibration curve (Table 8). No sample had a quantitative level of 4-methylguaiacol.

Target free form volatile phenols were quantitated by white wine calibration from three replicates of each red wine sample (Table 9). Note that 4-methylguaiacol and 3,5-xylenol were below limit of quantitation (LOQ) for all samples, and therefore are not included in the table. The slight decrease in concentration of guaiacol from the Milli-Q water calibration to the white wine calibrations (standard deviation = 0.82 and RSD = 9.35%) indicates the matrix effects that wine has on the quantitation.

Conclusion

Consumers tend to respond negatively to smoke-affected wines. Since there are no effective ways to remove smoke compounds from grapes or wines, smoke impact can be a major problem for a vineyard. This contamination can be a significant financial impact for the grape-grower, as no harvest would mean no income. There is also a reputational risk, not only for the grape-grower but for the region.[6] The Agilent SPME-GC/MS/MS method for the analysis of free-form volatile phenols associated with smoke impact allows for confident identification and reliable quantitation.

References

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2. Herve, E.; Price, S.; Burns, G. Free Guaiacol and 4-Methylguaiacol as Markers of Smoke Taint in Grapes and Wines: Observations from the 2008 Vintage in California. *ETS Laboratories*, **2011**.
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Table 8. Guaiacol levels identified in wine matrices.

Guaiacol	Franzia White Wine	Franzia Red Wine	CA Pinot Noir	OR Pinot Noir	Red Wine Sample
Average Concentration, n = 3 (ppb)	0.64	6.74	10.27	5.16	9.15
Standard Deviation	0.33	0.65	1.17	0.42	0.80
% RSD	51.80	9.57	11.40	8.13	8.72

Table 9. Average concentration (ppb) of targets identified in red wine samples.

Sample	Guaiacol	*o*-Cresol	Phenol	2,5-Xylenol	4-Ethylguaiacol	2-Ethylphenol	*p*-Cresol	*m*-Cresol	4-Ethylphenol	2,6-Dimethoxyphenol
Franzia Red Wine	6.32	0.41	2.73	<LOQ	0.09	<LOQ	1.61	0.38	< LOQ	0.77
CA Pinot Noir	9.97	1.90	5.58	0.23	0.22	0.01	0.75	0.68	0.08	1.05
OR Pinot Noir	4.68	2.05	6.20	16.23	10.81	<LOQ	1.73	1.44	24.81	0.60
Red Wine Sample	8.81	5.70	16.35	<LOQ	<LOQ	0.03	4.61	2.30	0.16	0.57

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