Application Note

Food Testing & Agriculture

SPME-GC/MS of 2,4,6-Trichloroanisole using an Agilent DVB/PDMS SPME Fiber

Author

Jessica Westland
Agilent Technologies Inc.

Abstract

Cork taint is an off-flavor problem in wine, mainly due to the presence of 2,4,6-trichloroanisole (TCA) in the cork stopper.[1] In addition to TCA, the presence of other chloroanisole compounds can also result in, or contribute to, cork taint. This study analyzed the levels of 2,4-dichloroanisole (DCA) and 2,4,6-TCA in a red wine sample using HS-SPME and GC/MS detection.

Introduction

There is growing concern in the wine industry about the quality of the products it manufactures, partly motivated by the increasing consumer awareness of quality issues. Consumers can detect different organoleptic defects. One of those is cork taint, which often has been chemically identified as 2,4,6-TCA. The perception limit of this compound is very low (close to 10 and 40 ng/L for white and red wines, respectively), so even at such low concentrations, its presence becomes a problem in wine quality.[2]

However, true cork taint in wine is rare. This denotation gives the idea that the origin of this defect is only the cork; however, there is evidence that the compounds causing cork taint may also appear from other sources.[2] There are many other causes that can explain the presence of TCA in wine. These include fungicides, biocides, herbicides, and wood preservatives containing chlorophenols, contamination coming from the cardboard used in the transport of the corks, use of hypochlorite as a cork bleaching agent, etc.

To avoid the economic losses due to this musty off-flavor, it is important to prevent the occurrence of this defect with an effective control of chloroanisoles. This control requires appropriate analytical methods, which must provide sensitivity and selectivity as well as good repeatability and recovery.

Among the various methods developed for the analysis of chloroanisoles in aqueous samples, gas chromatographic methods are most often used because of their high sensitivity and power of resolution.[1] This Application Note used gas chromatography coupled with mass-spectroscopy (GC/MS) for the analysis of 2,4-DCA and 2,4,6-TCA.

Experimental

Ten milliliters of red wine (Figure 1) was added to a 20 mL headspace vial containing ~4 g of NaCl. The addition of salt (NaCl) allows for the decrease of the partition coefficient between the liquid and gas phases, allowing more analytes to readily partition into the headspace. Twenty-five microliters of 10 ppm Methylated Haloacetic Acids Mixture (p/n PHM-552M) was spiked into the wine sample for analysis.

GC/MS analysis

2,4-DCA and 2,4,6-TCA were analyzed in a red wine sample using an Agilent 65 µm DVB/PDMS SPME fiber with a PAL RTC rail system. This was combined with an Agilent 7890B GC system, coupled with an Agilent 5977B High Efficiency Source GC/MSD (Figure 3).

Figure 1. Red wine sample used for extraction of 2,4,6-TCA with an Agilent DVB/PDMS SPME fiber.

Table 1. SPME headspace parameters.

SPME Headspace Parameters	
Script Name	ARROW-STD-V2.0
Tool	SPME 1
SPME Fiber Phase	65 µm DVB/PDMS; p/n 5191-5873 (Figure 2)
Incubation Time	30 minutes
Stirrer	Heatex Stirrer 1
Heatex Stirrer Speed (Agitation)	1,000 rpm
Heatex Stirrer Temperature (Extraction Temperature)	30 °C
Agitator	None
Sample Extract Time	30 minutes
Extraction Temperature	30 °C
Sample Vial Penetration Depth	40 mm
Sample Vial Penetration Speed	20 mm/s
Inlet Penetration Depth	40 mm
Inlet Penetration Speed	100 mm/s
Injection Signal Mode	Before fiber expose
Sample Desorption Time	3 minutes
Conditioning Port	SPMEArrowCond 1
Predesorption Conditioning Time	**Analytical Run:** 5 minutes **Precondition:** 30 minutes
Fiber Conditioning Station Temperature	250 °C
Post Desorption Conditioning Time	0 minutes
GC Cycle Time	5 minutes (set for sequence overlap)

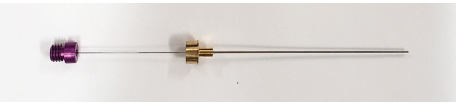

Figure 2. 65 µm DVB/PDMS (p/n 5191-5873).

Figure 3. The PAL RTC rail system combined with an Agilent 7890B GC and an Agilent 5977B GC/MSD.

GC/MS analysis

2,4-DCA and 2,4,6-TCA were analyzed using SPME headspace with a PAL RTC rail system. This was combined with an Agilent 7890B GC system, coupled with an Agilent 5977B High Efficiency Source GC/MSD (Figure 3).

Table 2. Agilent 7890B GC settings.

Agilent 7890B GC Settings	
Inlet Liner	Inlet liner, Ultra Inert, splitless, straight, 0.75 mm id (p/n 5190-4048)
Injection Mode/ Temperature	Splitless/220 °C
Oven Program	40 °C (hold 1 minute); 5 °C/min to 60 °C (hold 1 minute); 3 °C/min to 125 °C (hold 1 minute); 10 °C/min to 238 °C
Equilibration Time	0.5 minutes
Control Mode	Constant flow (1.2 mL/min)
Column	Agilent J&W HP-5ms Ultra Inert Intuvo GC column module, 15 m, 0.25 mm, 0.25 µm (p/n 19091S-431UI)
Septum Purge Flow Mode	Standard at 3 mL/min
Purge Flow to Split Vent	15 mL/min at 0.35 minutes
Agilent 5977B GC/MS Conditions	
Transfer Line	280 °C
Acquisition Mode	SCAN
Solvent Delay	0.5 minutes
Tune File	atune.u
Gain	1
MS Source Temperature	280 °C
MS Quad Temperature	150 °C

Results and discussion

DVB/PDMS fiber reproducibility

Eight replicate injections of spiked wine samples were performed on three different 65 µm DVB/PDMS fibers. Percent RSDs were calculated for each fiber, then averaged together. Each set of replications maintained %RSD values lower than 30%. Table 3 shows the averaged results. Figures 4 to 6 show the chromatographic data for the analysis.

Table 3. Compound %RSD results per DVB/PDMS fiber.

Compound	Fiber 01	Fiber 02	Fiber 03	Average
2,4-Dichloroanisole	4.41	27.28	5.75	12.48
2,4,6-Trichloroanisole	12.22	24.56	14.03	16.94

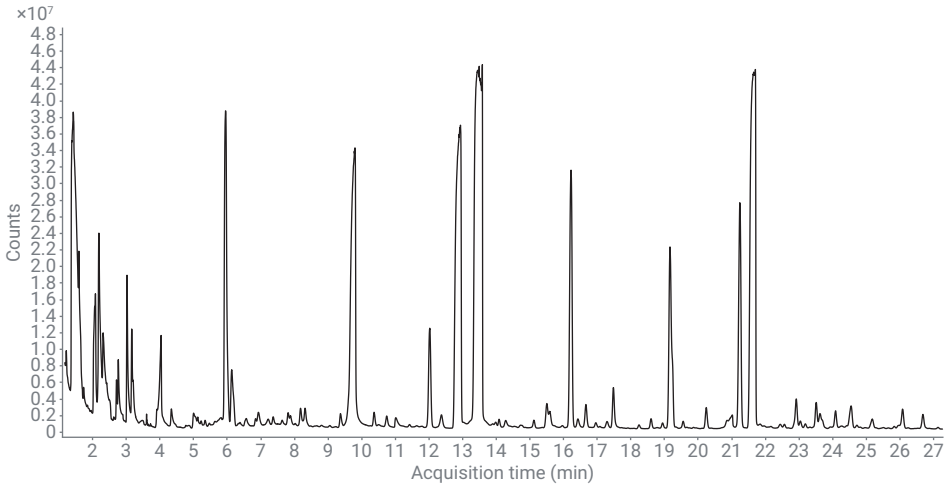

Figure 4. Scan chromatogram of blank red wine sample.

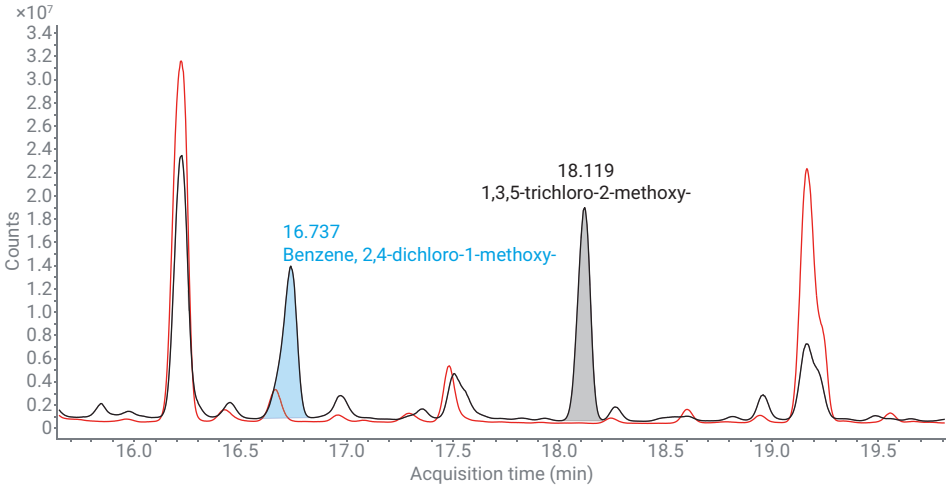

Figure 5. Overlay chromatograms of blank red wine sample (red trace) and DCA and TCA in a spiked red wine sample (black trace).

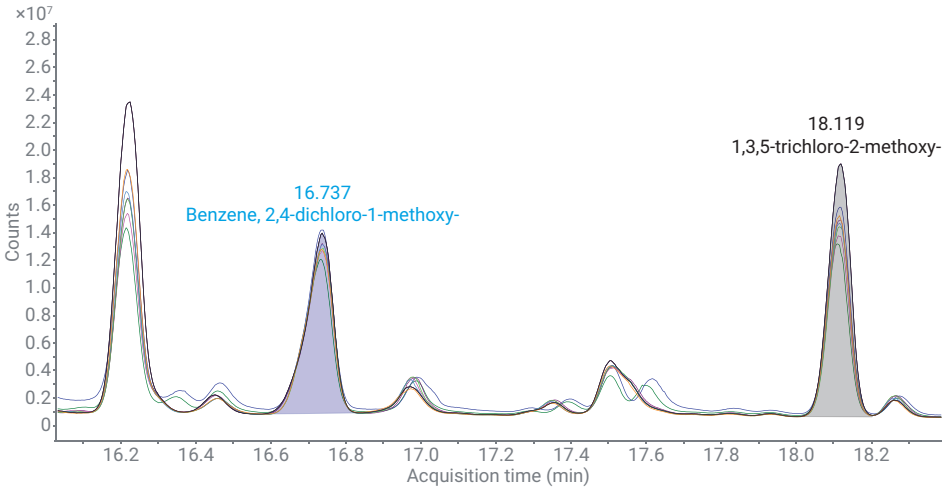

Figure 6. Overlay chromatograms of DCA and TCA in eight spiked red wine sample replicates from the same 65 µm DVB/PDMS SPME fiber.

Conclusion

The presence or absence of aroma compounds plays a vital role in the quality of food and beverages. In wine, aroma properties have a significant influence on the acceptance and appreciation of product.[3] However, when the aroma of wine has been affected with cork taint, the odor can provide an intense and distinct musty, moldy aroma. The TCA has been chemically identified as the compound that gives off this moldy aroma. The use of SPME-GC/MS with an Agilent 65 µm DVB/PDMS SPME fiber provides a reliable way to identify TCA in wine samples.

References

1. Özhan, D. *et al.* Determination of Chloroanisoles and Chlorophenols in Cork and Wine using HS-SPME and GC-ECD Detection. *Journal of the Institute of Brewing*, **2009**, *115,* 71—77, DOI:10.1002/j.2050-0416.2009.tb00346.x
2. Riu, M. *et al.* Determination of 2,4,6-Trichloroanisole in Wines by Headspace Solid-Phase Microextraction and Gas Chromatography–Electron-Capture Detection. *Journal of Chromatography A*, **2002**, *977(1)*, 1–8, ISSN 0021-9673, DOI:10.1016/S0021-9673(02)01276-1.
3. Azzi-Achkouty, S. *et al.* Headspace Solid-Phase Microextraction for Wine Volatile Analysis. *Critical Reviews in Food Science and Nutrition*, **2017**, *57(10)*, 2009-2020, DOI: 10.1080/10408398.2014.957379

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Printed in the USA, November 5, 2019
5994-1564EN

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