Using HS-SPME and GC/Triple Quadrupole MS for High Throughput Analysis of Haloanisoles in Wines at Sub-ng/L Levels

Application Note

Food

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Abstract

Haloanisole contamination causes cork taint, a musty off-aroma, in affected wines. Cork taint results in significant economic loss for the wine and allied industries every year. Using headspace-solid phase microextraction (HS-SPME) coupled to an Agilent 7890A GC and Agilent 7000B Triple Quadrupole GC/MS, we obtained a limit of quantification (LOQ) for TCA that is 0.5 ng/L in wine. LOQs for TeCA, TBA, and PCA are ≤ 1.0 ng/L. These LOQs are below the sensory threshold levels for these haloanisoles in wine. This method is automated, requiring only the addition of internal standards, and high throughput, with an extraction time of 10 minutes.

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Introduction

Haloanisoles (for example, 2,4,6-trichloroanisole (TCA), 2,3,4,6-tetrachloroanisole (TeCA), 2,3,4,5,6-pentachloroanisole (PCA), and 2,4,6-tribromoanisole (TBA) are responsible for musty aromas in many foods and beverages, even in trace levels. In wine, this contamination is frequently referred to as cork taint, affecting approximately 1 to 5% of wines on the market and resulting in significant losses in revenues. The major source of haloanisole contamination in wine is contaminated corks, although oak barrels and other winery-related sources are sometimes implicated [1].

Haloanisoles have very low sensory threshold levels, in the low ng/L range (~ 3 ng/L for TCA in wine), and therefore sensitive and specific analytical methods for the quantitative analysis of haloanisoles in wines are needed. Most wineries and cork suppliers have established detailed quality control analysis procedures including routine monitoring of cork lots and wine samples for haloanisole levels throughout processing and storage, which necessitate a high throughput method.

Many methods for haloanisole analysis use HS-SPME, but extraction times > 20 minutes limit the speed at which the analysis can be completed in a production environment. In addition, many methods also do not use stable isotope internal standards to assure accurate quantitative analysis. Tandem MS (MS/MS) is often used for targeted analysis of wine components, due to its low limits of detection (LODs) (sensitivity) and high selectivity for the analytes of interest. This application note describes a published method using HS-SPME and stable isotope internal standards for a rapid, highly reproducible, and accurate MS/MS analysis of haloanisoles on the Agilent 7890A GC, with an Agilent 7000B Triple Quadrupole GC/MS. It provides LODs and LOQs for TCA, TeCA, PCA, and TBA that are ≤ 1 ng/L [1].

Experimental

Standards and Reagents

Haloanisole standards were purchased, and stock solutions prepared as previously described [1]. A model wine was also prepared as described previously and used for initial evaluations and preparation of calibration standards.

Five commercial wines (two reds and three whites) were obtained locally and used for the analyte recovery experiments. In addition, five tainted wines reported by consumers to have taint aromas were obtained from a local testing laboratory [1].

Instruments

This method was developed on an Agilent 7890A gas chromatograph equipped with a SPME injection liner in the inlet, coupled to an Agilent 7000B Triple Quadrupole GC/MS. A Gerstel MPS2 autosampler was mounted on the 7890A GC to perform the headspace sample extractions. The GC/MS/MS instrument run conditions are listed in Table 1.

Table 1. Gas Chromatograph and Mass Spectrometer Conditions

GC run conditions	
Analytical column	30 m × 0.25 mm, 0.25 µm DB-5 (p/n 122-5032)
Inlet	Splitless, equipped with an SPME injection port liner
Injection	Splitless; split flow opening at 1.2 minutes, flow rate of 50 mL/min for 3 minutes, when the flow was changed to 20 mL/min
Carrier gas	Helium, constant flow, 1.2 mL/min
Oven program	40 °C for 0 minutes 30 °C/min to 280 °C 3 minutes hold
Transfer line temperature	280 °C
MS run conditions	
Solvent delay	5 minutes
EMV gain	15
Acquisition parameters	EI, multiple reaction monitoring (MRM)
Scan widths	±1.2 *m/z*
Quench gas	Helium at 2.25 mL/min
Collision gas	Nitrogen at 1.5 mL/min

Sample preparation

Wine samples (10 mL) were transferred into 20-mL amber glass sample vials, to which 50 µL of the stock internal standard solution was added. The final concentration of internal standards was 5.0 ng/L for [$^2H_5$] TCA and [$^{13}C_6$] PCA and 10 ng/L for [$^2H_5$] TBA in the sample. Prior to extraction, the samples were agitated at 40 °C and 500 rpm for 5 minutes. Extraction of samples was performed immediately by inserting a preconditioned 100 µm PDMS SPME fiber into the headspace of the vial for 10 minutes while agitating at 250 rpm. The fiber was then thermally desorbed for the entire oven cycle time (11 minutes) in the GC inlet at 280 °C to prevent analyte carryover between samples. To prevent contamination and loss of sample, the fiber was always either in the inlet or extracting a sample. All analyses were performed in triplicate.

Analysis parameters

The parameters used in the analysis of four haloanisoles are shown in Table 2.

Table 2. Analysis Parameters

Analyte/internal standard	Retention time (min)	Transition (*m/z*)	Collision energy (V)
Trichloroanisole (TCA)	5.21	210 → 195*	10
		212 → 197†	10
[$^2H_5$] Trichloroanisole	5.20	215 → 197	10
		217 → 199	10
Tetrachloroanisole (TeCA)	6.10	246 → 203	25
		231 → 203	15
Tribromoanisole (TBA)	6.50	344 → 329	10
		346 → 331	10
[$^2H_5$] Tribromoanisole	6.48	351 → 333	15
		349 → 331	15
Pentachloroanisole (PCA)	6.91	265 → 237	10
		280 → 237	25
[$^{13}C_6$] Pentachloroanisole	6.91	286 → 242	25
		286 → 271	10

* For all transitions listed, first number refers to quantifier transition

† For all transitions listed, second number refers to qualifier transition

Source: Hjelmeland *et al.* [1].

Results and Discussion

Linearity, LOD, and LOQ

The results in Table 3 demonstrate linear responses for each of the haloanisoles in a model wine matrix. LODs and LOQs obtained were substantially lower than the respective sensory thresholds. Under these analysis conditions, the least volatile analyte, TBA, had the highest LOD and LOQ. This method provides a trade-off between optimal sensitivity and rapid throughput enabled by short extraction times. The LODs and LOQs obtained were lower than those typically reported in previously published studies for HS-SPME analysis of haloanisoles in water, wine, and cork extracts [2,3]. These previous studies also used HS-SPME extraction times of $\geq$ 25 minutes and single quadrupole selected ion monitoring (SIM) detection.

Table 3. Haloanisole Calibration, Linearity, LODs and LOQs in Model Wines

Analyte	Standard curve range (ng/L)	Correlation coefficient (R)	LOQ (ng/L)	LOD (ng/L)	Threshold* (ng/L)
TCA	0.10–50	0.9992	0.50	0.10	3.0
TeCA	0.10–50	0.9997	0.10	< 0.10†	15
PCA	0.10–50	0.9996	0.25	0.10	3.0
TBA	0.50–50	0.9991	1.0	0.50	10,000

* Sensory threshold in wine

† No standards <0.10 ng/L were analyzed

Source: Hjelmeland *et al.* [1].

Recovery and reproducibility

Using our method, recoveries for haloanisole in a variety of red and white wine matrices were between 90% and 110%, with relative standard deviations (% RSD) less than 10% in most cases (Table 4). Similar values have been reported [2,4]. While it has been reported that matrix interferences can limit sub ng/L detection, our tandem MS approach, combined with HS-SPME, did not reveal matrix interferences in the wines evaluated. When background levels were observed, haloanisole contamination was identified as the source, based on ion ratios. Low levels of haloanisoles are not unexpected in a commercial winery setting, as it is extremely difficult to eliminate all haloanisole contamination from air, water, and glassware. Contamination of the components of the analytical system must also be monitored. For example, plastic components must be cleaned after running high calibration standards, and after analysis of concentrations > 100 ng/L the front end of the column (~2.5 cm) should be cut off to prevent carryover [1].

Table 4. Haloanisole Spiked Recovery and Reproducibility in Wine

		Measured value				
Analyte	**Wine**	**Amount spiked (ng/L)**	**In blank (ng/L)**	**After spike (ng/L)**	**Spiked recovery (%)**	**RSD (%)**
TCA	Petite syrah	1.0	nd*	0.94	94	2.5
		5.0		4.7	94	4.7
	Sauvignon blanc	1.0	nd	0.93	93	11
		5.0		4.8	97	2.2
	Gewürztraminer	1.0	< LOQ	1.0	97	17
		5.0		5.0	99	4.8
	Riesling	1.0	nd	1.0	103	9.1
		5.0		4.9	98	5.5
	Cabernet sauvignon	1.0	nd	1.1	106	6.1
		5.0		5.1	102	1.3
TeCA	Petite syrah	1.0	0.7	1.1	109	13
		5.0		5.5	110	4.2
	Sauvignon blanc	1.0	nd	1.0	98	3.7
		5.0		4.6	91	2.8
	Gewürztraminer	1.0	nd	1.0	103	5.5
		5.0		5.2	104	9.9
	Riesling	1.0	nd	1.0	100	4.1
		5.0		5.2	104	3.3
	Cabernet sauvignon	1.0	nd	1.0	102	2.0
		5.0		5.4	108	1.3
PCA	Petite syrah	1.0	nd	1.0	96	9.0
		5.0		5.1	103	3.6
	Sauvignon blanc	1.0	nd	1.0	105	8.9
		5.0		5.2	103	3.2
	Gewürztraminer	1.0	nd	1.0	105	2.7
		5.0		5.3	106	2.6
	Riesling	1.0	nd	1.1	108	1.9
		5.0		4.8	97	2.2
	Cabernet sauvignon	1.0	nd	1.1	109	6.0
		5.0		5.3	107	4.7
TBA	Petite syrah	1.0	nd	1.1	109	15
		5.0		5.3	107	9.7
	Sauvignon blanc	1.0	nd	1.0	104	11
		5.0		5.1	102	4.1
	Gewürztraminer	1.0	nd	1.0	103	3.2
		5.0		5.1	102	2.3
	Riesling	1.0	nd	1.0	104	9.7
		5.0		5.3	106	0.4
	Cabernet sauvignon	1.0	nd	1.1	106	7.7
		5.0		5.0	101	7.3

* not detected; below LOD
Source: Hjelmeland *et al.* [1].

Analysis of tainted wines

Analysis of wines reported by consumers to be tainted revealed that TCA was the principal haloanisole detected at levels near or above reported sensory thresholds (Table 5, Figure 1). The other haloanisoles were not detected, or were present at approximately 10 times lower concentrations than TCA.

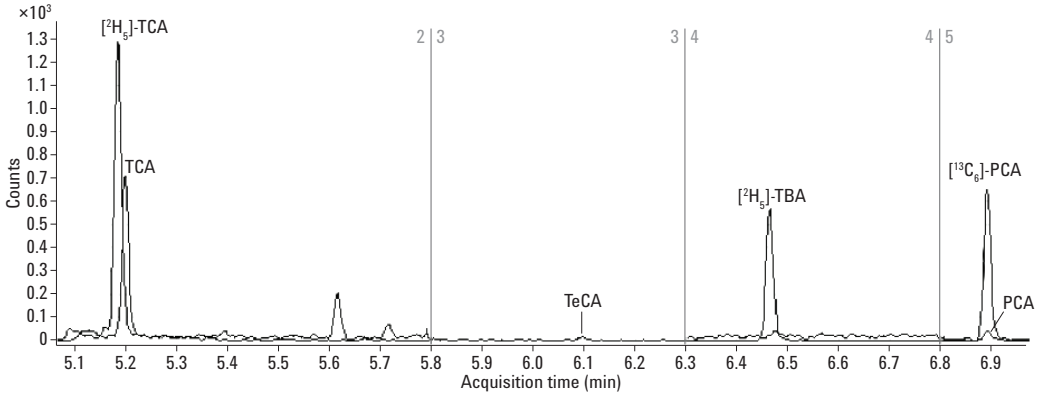

Figure 1. Example chromatogram of consumer complaint wine A (Table 6). The calculated TCA level was 2.3 ng/L. Source: Hjelmeland et al. [1].

Table 5. Haloanisole Concentrations in Three Tainted Wines†

	Measured concentration (ng/L) and (%RSD)		
Analyte	**Wine A**	**Wine B**	**Wine C**
TCA	2.3 (4.5)	9.9 (3.4)	6.8 (5.6)
TeCA	0.18 (6.9)	0.16 (0.7)	0.17 (3.5)
PCA	0.41 (18)	nd*	0.26 (9.0)
TBA	nd*	0.65 (1.8)	0.73 (1.8)

† Obtained from a commercial laboratory

* not detected; below LOD

Source: Hjelmeland *et al.* [1].

Conclusion

A rapid and highly reproducible method for analysis of TCA, TeCA, PCA, and TBA in wines has been developed using HS-HPME and MS/MS analysis on the Agilent 7000B Triple Quadrupole GC/MS. LODs and LOQs for TCA, TeCA, and PCA were well below 1 ng/L, and were ≤ 1 ng/L for TBA. These levels are below the reported sensory thresholds, and may be important for monitoring winery processes over time to ensure that no sources of contamination that could taint the wines during processing and storage exist. The total HS-SPME extraction time for the optimized method was 15 minutes, including a 5-minute pre-agitation. The GC analysis time was 11 minutes.

References

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