ExtraTECH
- ANALYTICAL SOLUTIONS -

# Room temperature and sensitive analysis of haloanisoles in wine using Vacuum-assisted headspace SPME with GC/ECD


## ABSTRACT

Haloanisole contamination causes a musty/moldy off-aroma in affected wines, and results in significant economic loss for the wine and allied industries every year. The extremely low human sensory thresholds for these compounds require the use of highly sensitive analytical methods to detect them at odor threshold concentrations or lower. Using vacuum-assisted headspace solid-phase microextraction (Vac-HS-SPME) sampling at room temperature followed by GC-ECD we developed a quick and sensitive procedure for the analysis of haloanisoles from wine. A comparative study between Vac-HS-SPME and regular HS-SPME was carried out and their greenness was evaluated using AGREEprep metric tool.


## INTRODUCTION

Haloanisoles are well-known for creating a musty/moldy off-aroma in wines that is rejected by consumers, causing important economic losses for the wine industry. The defect is known as "cork taint" and is attributed to the cork stopper; though the problem can be widespread and affect the barrels, pipes and beams of a whole cellar. The main compounds responsible for the musty odor in wines are 2,4,6-trichloroanisole (TCA), 2,3,4,6-tetrachloroanisole (TeCA), pentachloroanisole (PCA) and 2,4,6-tribromoanisole (TBA), with TCA being detected in close to 80% of the positive samples. Because of the extremely low human sensory thresholds for these compounds (ppt level), highly sensitive analytical methods are needed to detect the haloanisoles at threshold concentrations or lower.

Headspace solid-phase microextraction (HS-SPME) followed by gas chromatography (GC) coupled - mass spectrometry or electron capture detection (ECD) is widely used for the detection and quantification of haloanisoles in wine. Reported methods require long extraction times or elevated SPME sampling temperatures to address the problem of low headspace concentrations. An alternative way to improve HS-SPME extraction efficiencies is to sample the headspace under reduced pressure conditions using the vacuum-assisted HS-SPME (Vac-HS-SPME) approach.

In this application, a fast, room temperature and sensitive Vac-HS-SPME method is proposed for the determination of haloanisoles in wine. A comparative study between Vac-HS-SPME and regular HS-SPME was carried out to demonstrate the benefits of adopting the vacuum approach. The proposed Vac-HS-SPME procedure was also used for the determination of haloanisoles in several bottled red wines. Finally, the greenness of the method was assessed using AGREEprep metric tool.

## EXPERIMENTAL

Table 1 and Table 2 describe the final optimized Vac-HS-SPME and standard HS-SPME methods, respectively. Table 3 gives details on the GC-ECD method. Optimization was performed using a synthetic wine solution prepared by dissolving L(+)-tartaric acid (5 g/L) in a hydroalcoholic solution (13% v/v ethanol) and the pH of the solution was adjusted to 3.5. Calibration curves were constructed in synthetic wine and red wine samples via external calibration.

*Table 1. Optimized Vac-HS-SPME method.*

| | |
|---|---|
| **Sample:** | 5 mL wine sample in 20 mL crimp top vial; ExtraTECH Vac-closure (PN: 20-101) |
| **Air- evacuation** | 1 min before sample introduction, pumping unit with 7 mbar ultimate vacuum |
| **SPME Fiber:** | PDMS/DVB, 65 µm film |
| **Incubation:** | 10 min, 25 °C, agitation |
| **Extraction:** | 30 min, 25 °C, agitation |

*Table 2. Optimized regular HS-SPME method.*

| | |
|---|---|
| **Sample:** | 5 mL wine sample |
| **Fiber:** | PDMS/DVB, 65 µm film |
| **Incubation:** | 10 min, 55 °C, agitation |
| **Extraction:** | 30 min, 55 °C, agitation |

*Table 3. GC-ECD method.*

| | |
|---|---|
| **Column:** | DB5MS (30 m x 0.250 mm i.D., 0.25 µm) |
| **Oven:** | 90 °C (5 min), 20 °C/min to 280 °C (5 min) |
| **Inj. Temp.:** | 270 °C |
| **Carrier Gas:** | Helium, 1 mL / min constant flow |
| **Detector:** | ECD |
| **Injection:** | Splitless, 5min |
| **Desorption:** | 15 min at 270 °C |



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## RESULTS

The two SPME procedures necessitated the optimization of sampling temperature and extraction time and the optimum values found were: (i) for regular HS-SPME 30 min sampling at 55 °C and (ii) for Vac-HS-SPME 30 min sampling at 25 °C. The analytical performances of the optimized Vac- and regular HS-SPME procedures were evaluated using simulated wine as solvent. Table 4 summarizes the main analytical parameters. The calibration curves showed good linearity in a wide concentration range. The obtained relative standard deviations (RSDs) ranged from 4.0 to 10.9% with Vac-HS-SPME and from 4.8 to 10.6% with regular HS-SPME. Limits of detection (LODs) were estimated according to three times the signal-to-noise-ratio (S/N). Based on the results, an overall superior analytical performance was recorded with Vac-HS-SPME compared to regular HS-SPME, even though Vac-HS-SPME sampling proceeded at a much lower temperature (25 °C versus 55 °C with the standard HS-SPME).

*Table 4. Haloanlisole calibration, linearity, LOD in synthetic wine*

| | Vac-HS-SPME (25 °C) | | | HS-SPME (55 °C) | | |
|---|---|---|---|---|---|---|
| | Linearity range (ng/L) | $r^2$ | LOD (ng/L) | Linearity range (ng/L) | $r^2$ | LOD (ng/L) |
| TCA | 0.25-25 | 0.998 | 0.16 | 0.75-25 | 0.998 | 0.44 |
| TeCA | 0.25-25 | 0.998 | 0.18 | 0.75-25 | 0.999 | 0.37 |
| PCA | 0.25-25 | 0.998 | 0.19 | 0.75-25 | 0.999 | 0.26 |
| TBA | 0.25-25 | 0.994 | 0.13 | 0.75-25 | 0.997 | 0.66 |

### Analysis of real wine samples

The analytical performance of the optimized Vac-HS-SPME was also tested in real wine samples. The resulting calibration curves showed good linearity between 1.5 and 25 ng/L, and the coefficients of determination were 0.997, 0.997, 0.991 and 0.991 for TCA, TeCA, PCA and TBA respectively (n=5). LODs were estimated according to S/N=3 and were 0.43, 0.45, 0.31 and 0.64 ng/L for TCA, TeCA, PCA and TBA respectively. As expected, the limits of detection and quantification were affected due to the noise level increase and analyte interaction with matrix.

Finally, twelve bottled red wines were analyzed using the proposed Vac-HS-SPME. In one wine sample TCA was detected (Figure 1). The TCA concentration in the positive sample was calculated using standard addition method and was 3.2 ± 0.2 ng/L (n=3). This value was within the perception level for TCA (ranging between 0.03 to 10.0 ng/L) and below the 10 ng/L concentration threshold where a defect in wine is produced.

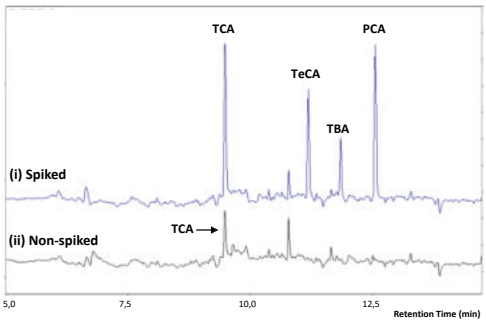


*Figure 1. GC-ECD analysis of spiked and non-spiked wine after Vac-HS-SPME sampling*

### Greenness of the methods

The greenness of the Vac- and standard HS-SPME methods were then assessed in the manual (Fig. 2(a)) or automated (Fig. 2(b)) mode using AGREEprep metric tool. The SPME methods had the same input values in almost all criteria except and criterion 8 (energy consumption) where the standard approach requires heating the sample at 55 °C (assumed 400 Wh/sample) whereas in Vac-HS-SPME heating is not applied (assumed 20 Wh/sample; agitation only). In automated method criterion 7 (integration and automation) was also different with Vac-HS-SPME assigned as semi-automated and HS-SPME as automated. The higher energy consumption with the regular HS-SPME procedure is reflected by the lower final score compared to Vac-HS-SPME in the manual or automated modes. This assessment shows that Vac-HS-SPME sampling is a greener approach.

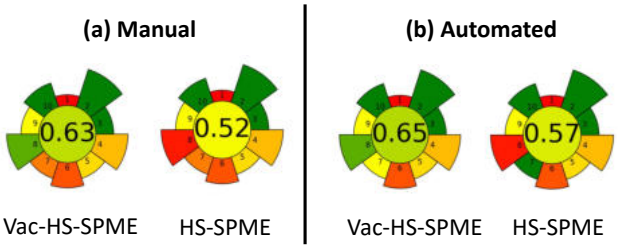


*Fig. 2. The results of the AGREEprep assessment of the (a) manual Vac and standard HS-SPME-GC-ECD and (b) automated Vac and standard HS-SPME-GC-ECD methods*

## CONCLUSIONS

The proposed Vac-HS-SPME procedure is a fast, room temperature and sensitive method for determining haloanisoles in wine samples. The optimum sampling time under each pressure condition was 30 min and sampling under vacuum excluded heating the sample at elevated temperatures as seen in the standard method. Sampling at room temperature is particularly advantageous to maintain the sample composition and minimize the evaporation of potential volatile components acting as matrix interferences during real wine sample analysis.

**REFERENCE:** M. Vakinti et al., J. Chromatogr. A 1602 (2019) 142–149 (https://doi.org/10.1016/j.chroma.2019.03.047)