Application note | 001411

Food analysis

Determination of haloanisoles in wine by HS-SPME Arrow and GC-MS/MS

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Keywords

Haloanisoles, trichloroanisole, TCA, wine, gas chromatography-mass spectrometry, GC-MS, triple quadrupole, TSQ 9610 mass spectrometer, NeverVent AEI, solid phase micro-extraction, SPME, SPME Arrow, TriPlus RSH SMART autosampler

Goal

The aim of this application note is to demonstrate the performance of the Thermo Scientific™ TSQ™ 9610 triple quadrupole mass spectrometer coupled to solid phase microextraction with Arrow technology (SPME Arrow) for the determination of haloanisoles as contaminants in wines.

Introduction

Haloanisoles (2,4,6-trichloroanisole (TCA); 2,3,4,6-tetrachloroanisole (TeCA); pentachloroanisole (PCA); and tribromoanisole (TBA)) are the main source of contamination in wine, resulting in unwanted musty sensory characteristics. Haloanisole contamination can originate from cork stoppers, barrels, and other factors in a winery; therefore, the determination of these compounds is critical to minimize the economic losses associated with producing tainted wines.

As the sensory threshold for these compounds falls in the low $ng \cdot L^{-1}$ range, sensitive analytical methods are key for both screening and quantitative analysis. Gas chromatography coupled to triple quadrupole mass spectrometry (GC-MS/MS) is the preferred technique for this application as it offers the sensitivity required for reliable detection of these compounds, combined with the selectivity to discriminate between the target compounds and other potentially interfering compounds in a complex matrix

such as wine. Although various sample preparation techniques have been reported,[1] headspace sampling offers the advantage of minimal sample preparation and fast turnaround combined with expanded sensitivity when using solid-phase microextraction (HS-SPME) sampling. SPME[2] has proven to be an effective alternative to static headspace sampling (SHS), as it combines analyte extraction and enrichment in a single step, consequently allowing lower detection limits to be achieved. It consists of a fiber coated with an organic solid phase that, when exposed to the sample, extracts and concentrates the analytes using selective absorptive/adsorptive processes, providing improved extraction efficiency and superior sensitivity. The fiber can be exposed in the vapor phase above the liquid or solid matrix (headspace-SPME) or directly immersed in the liquid sample (direct immersion-SPME), offering the flexibility to analyze several matrices with one single solution. Recent development introducing the SPME Arrow technology offers additional advantages such as superior extraction efficiency and higher mechanical robustness.[3]

In this study, the performance of the HS-SPME Arrow sampling technique was evaluated for the determination of haloanisoles in wine. GC-MS/MS was used to ensure appropriate selectivity and sensitivity for matrix samples using selected reaction monitoring (SRM) acquisition mode. Additionally, the SPME workflow is fully integrated and controlled by the Thermo Scientific™ Chromeleon™ Chromatography Data System software offering a seamless workflow from sample extraction to data acquisition and reporting.

Experimental

In the experiments described in this note, a Thermo Scientific TSQ 9610 triple quadrupole mass spectrometer with a Thermo Scientific™ NeverVent™ Advanced Electron Ionization (AEI) ion source was coupled to a Thermo Scientific™ TRACE™ 1610 gas chromatograph (GC) equipped with a Thermo Scientific™ iConnect™ Split/Splitless (iC-SSL) injector and a Thermo Scientific™ TriPlus™ RSH SMART autosampler with SPME Arrow configuration. Chromatographic separation was achieved on a Thermo Scientific™ TraceGOLD™ TG-WaxMS capillary column, 30 m × 0.25 mm × 0.25 µm (P/N 26088-1421). A Thermo Scientific™ SMART SPME Arrow fiber with 100 µm PDMS coated phase (P/N 36SA10P1-SM) allowed for effective enrichment of the analytes of interest in only 15 minutes at 40 °C. The incubation temperature was selected considering the partition coefficient of the analytes as temperature is a controlling factor of the kinetics of the equilibrium between the headspace and the matrix. During method development, four incubation and extraction temperatures were investigated, covering 40, 50, 60, and 70 °C. The lowest temperature provided the most intense peak areas for analysis of TCA, whereas increased extraction temperatures led to a decrease of the distribution constant between the fiber solid phase and the sample headspace (K value) for TCA and TeCA, therefore reducing the enrichment efficiency for these compounds.

The overlapping capability of the TriPlus RSH SMART autosampler allows the execution of the extraction/enrichment step during the chromatographic run of the previous samples, ensuring a shorter cycle time for high sample throughput. Moreover, the autosampler provides an additional layer of reliability and confidence in the analytical results thanks to the automatic SMART fibers identification and usage tracking capabilities for a more efficient management of the autosampler consumables. Detailed HS-SPME, GC-MS/MS parameters as well as a complete list of the target compounds are reported in Tables 1 and 2, respectively.

Table 1A. GC-MS/MS experimental conditions for the analysis of haloanisoles

TRACE 1610 GC and TSQ 9610 triple quadrupole MS/MS parameters	
Inlet module and mode	SSL, splitless
Liner	SPME Arrow Liner, 1.7 mm ID
Inlet temperature (°C)	260
Splitless time (min)	3.5
Septum purge mode, flow (mL/min)	Constant, 5
Carrier gas, mode, flow (mL/min)	He, constant flow, 1.2
Oven temperature program	
Temperature 1 (°C)	40
Hold time (min)	3.5
Temperature 2 (°C)	150
Rate (°C/min)	35
Temperature 3 (°C)	160
Rate (°C/min)	15
Temperature 4 (°C)	250
Rate (°C/min)	20
Hold time (min)	3.2
GC total run time (min)	15
TSQ 9610 triple quadrupole MS/MS parameters	
Ion source	Advanced Electron Ionization (AEI)
Transfer line temperature (°C)	250
Source temperature (°C)	270
Ionization mode	EI
Electron energy (eV)	50
Emission current (µA)	10
Acquisition mode	Selected reaction monitoring (SRM)
Chromatographic column:	
TraceGOLD TG-WaxMS (P/N 26088-1421)	30 m × 0.25 mm × 0.25 µm

Table 1B. HS-SPME experimental conditions for the analysis of haloanisoles

TriPlus RSH SMART autosampler – SPME Arrow parameters	
Fiber	SMART SPME Arrow PDMS (P/N 36SA10P1-SM)
Coating phase thickness (µm)	100
Coating phase lenght (mm)	20
Incubation time (min)	5
Incubation and extraction temperature (°C)	40
Extraction time (min)	15
Analysis time (min)	23
Fiber conditioning temperature (°C)	280
Fiber pre-conditioning time (min)	0
Fiber post-conditioning time (min)	8
Fiber depth in vial (mm)	30
Fiber depth in injector (mm)	70
Desorption time (min):	3

Table 2. List of targeted haloanisoles, retention times (RT, min), SRM precursor and product ions (*m/z*), and collision energies (eV). Quantifier transitions are marked in bold.

Target analyte	RT (min)	Precursor ion (*m/z*)	Product ion (*m/z*)	Collision energy (eV)
2,4,6-trichloroanisole (TCA)	9.24	**212**	**197**	10
		210	195	10
		197	169	10
2,3,4,6-tetrachloroanisole (TeCA)	10.62	**246**	**231**	10
		203	143	20
		229	201	10
		231	203	10
Tribromoanisole (TBA)	11.56	**329**	**301**	10
		331	303	10
		344	329	10
		346	331	10
Pentachloroanisole (PCA)	11.80	**237**	**143**	20
		263	235	10
		265	237	10
		280	237	20

Data acquisition, processing, and reporting

Data were acquired, processed, and reported using Chromeleon CDS software, version 7.3. Integrated instrument control ensures full automation of the entire SPME workflow from sample incubation to analyte desorption combined with an intuitive user interface for data analysis, processing, customizable reporting, and storage in compliance with Title 21 of the Code of Federal Regulations Part 11 (Title 21 CFR Part 11).

Standard and sample preparation

2,4,6-trichloroanisole (TCA); 2,3,4,6-tetrachloroanisole (TeCA); tribromoanisole (TBA); and pentachloroanisole (PCA) standard solutions were purchased from LGC (Teddington, UK). Tartaric acid (≥99.5%), hydrochloric acid (37%), sodium chloride (NaCl, ≥99%), acetone (for residue analysis, 99.9%), ethanol (absolute, 99.9%), and HPLC-MS grade water were purchased from Fisher Scientific. The complete list of the P/Ns can be found in Appendix A.

Standard preparation

A model wine (13% ethanol, 5g/L tartaric acid, final pH=3.5)[4] was prepared and used to dilute the pure standards.

Standard solutions were diluted in acetone to a final concentration of 1,000 ng/L. This stock solution was further diluted in model wine to obtain seven calibration solutions ranging from 1 to 250 ng/L. The calibration solutions were then used to prepare wine matrix-matched calibration standards ranging from 0.1 to 25 ng/L. A 10 mL aliquot of each calibration standard was transferred into 20 mL headspace vials (P/N 6ASV20-1, caps P/N 6PMSC18-ST2). Each calibration level was prepared in duplicate.

NaCl (2 g) was added to the vials to generate a salting out effect and improve the extraction efficiency of the target compounds.

Sample preparation for determination of haloanisoles in wine samples

Samples of red wine (Sangiovese, Merlot) and white wine (Trebbiano) were purchased at a local retailer. An aliquot (10 mL) was transferred into 20 mL headspace vials and 2 g NaCl were added prior the analysis.

Wine matrix-matched calibration solutions as well as wine samples were used to assess recovery, method linearity, sensitivity, repeatability, and quantitative performance stability.

Results and discussion

Chromatography

Wine is a highly complex matrix containing a non-volatile fraction, including polyphenolic compounds, proteins, and carbohydrates, and a volatile fraction, which includes hundreds of flavor and aroma compounds with a broad variability of concentrations from few ng/L to hundreds of mg/L.[5] In addition, the composition of the matrix differs from wine to wine and includes components other than the haloanisols targeted here. For example, TCA is commonly detected using ions at the expected *m/z* ratios of 210 or 195, however, there are potentially interferences producing ions of similar mass when single quadrupole mass spectrometry is used, thus requiring highly efficient separations to chromatographically resolve the target compounds from the matrix interferences. HS-SPME sampling combined with the

SRM acquisition mode represents an ideal solution to remove the matrix interferences and to provide an extra selectivity for confident detection and accurate quantitation of trace level compounds in complex matrices. The Thermo Scientific™ AutoSRM™ software was used to automate and optimize the SRM transitions. The SIMBridge™ feature allowed easy importing of the list of transitions into the Chromeleon CDS method editor for fast and error-free setup of the acquisition list. As an example, the total ion chromatogram (TIC) acquired in EI, full-scan (FS) mode (*m/z* 50-500) for red wine (Sangiovese and Merlot) and white wine (Trebbiano) samples, as well as the SRM acquisition of Sangiovese wine sample spiked at 0.25 ng/L with all haloanisoles under investigation are shown in Figure 1. Baseline separation (R_s > 2) and Gaussian peak shapes (asymmetry factor As = 1.0) were achieved for the investigated compounds.

Analytes recovery

Recovery was assessed by preparing three spiking solutions at 2.5, 25, and 50 ng/L in model wine. The spiking solutions were then used to spike three Sangiovese samples at final concentrations of 0.25, 2.5, and 5.0 ng/L. Each sample was prepared in duplicate. Calculated recoveries were between 90% and 105% of the spiked concentration for the investigated analytes, as reported in Table 3.

Linearity and method detection limit (MDL)

Calibration curves ranging from 0.10 to 25 ng/L (seven calibration levels) were used to assess method linearity and detection limits. Linear calibration was plotted considering the average response of each duplicated calibration level. Adequate linearity was obtained with coefficient of determination (R^2) ≥ 0.997 and average calibration factor %RSD (AvCF %RSD) ≤ 8.2% for all investigated compounds (Table 4). Full range (0.1–25 ng/L) and zoomed range (0.1–5.0 ng/L) calibration curves for the investigated haloanisoles are reported in Figure 2.

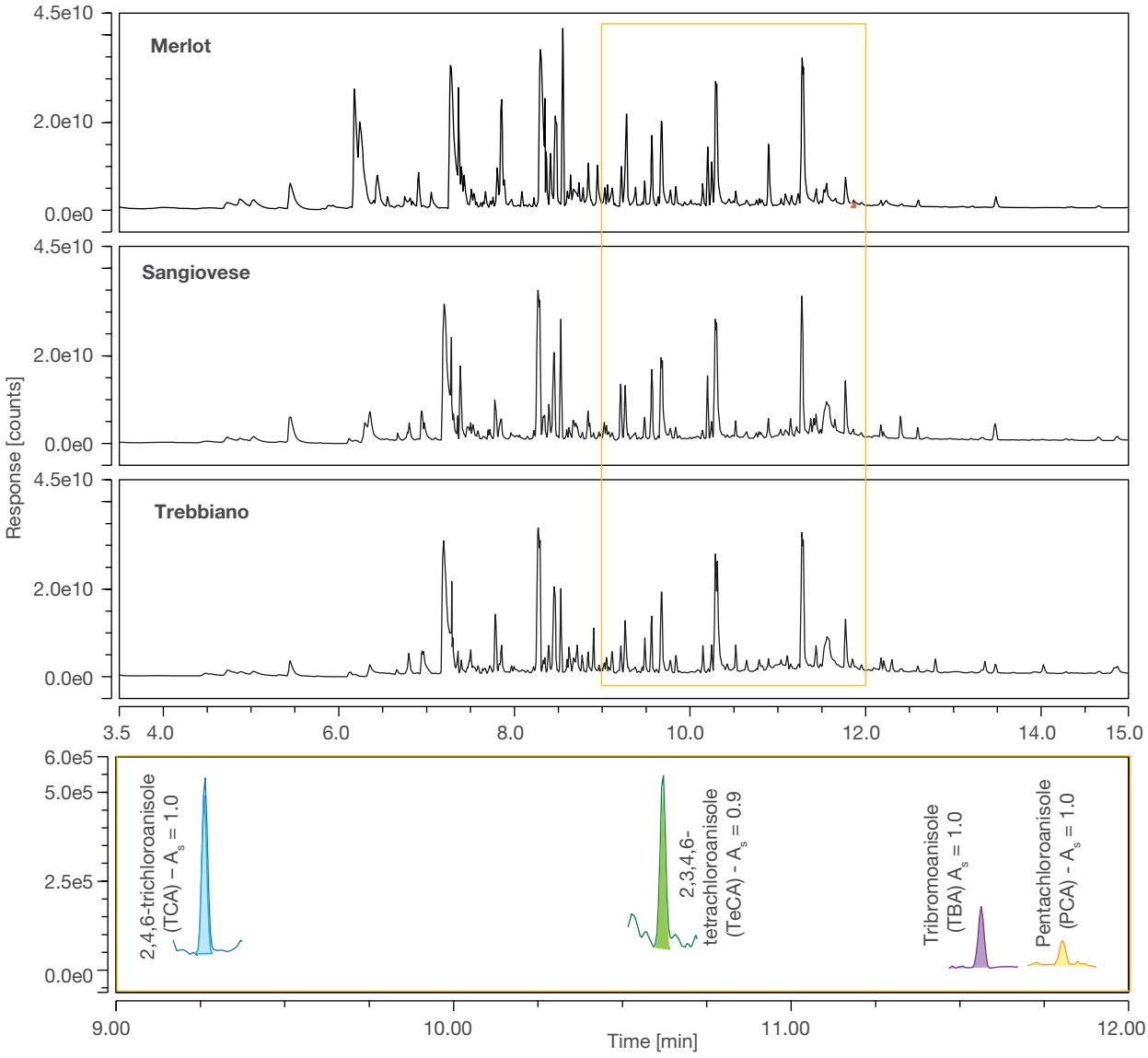

Figure 1. TIC (FS: *m/z* 50–500, upper traces) for red and white wine samples and SRM acquisition (yellow framed bottom trace) for a Sangiovese sample spiked at 0.25 ng/L. A_s values are annotated.

Table 3. Calculated recoveries of target compounds (%) from Sangiovese wine samples spiked at 0.25, 2.5, and 5.0 ng/L. Calculated recovery was 90–105%.

Target analyte	RT (min)	Spiked concentration (ng/L)	Calculated concentration (ng/L)		Recovery (%)	
			Sample 1	Sample 2	Sample 1	Sample 2
TCA	9.24	0.3	0.23	0.23	94	90
		2.5	2.47	2.38	99	95
		5.0	4.90	4.65	98	93
TeCA	10.62	0.3	0.23	0.23	94	91
		2.5	2.47	2.29	99	92
		5.0	4.83	4.66	97	93
TBA	11.56	0.3	0.24	0.25	95	99
		2.5	2.53	2.42	101	97
		5.0	5.00	4.80	100	96
PCA	11.80	0.3	0.26	0.25	105	99
		2.5	2.44	2.35	98	94
		5.0	4.68	4.84	94	97

Table 4. Coefficient of determination (R^2), average calibration factor (AvCF) %RSD, calculated MDL (ng/L) and LOQ (ng/L)

Target analyte	RT (min)	Coefficient of determination (R^2)	AvCF %RSD	Calculated MDL (ng/L)	LOQ (ng/L)
TCA	9.24	0.997	8.2	0.03	0.11
TeCA	10.62	0.998	7.4	0.04	0.14
TBA	11.56	0.998	6.7	0.07	0.23
PCA	11.80	0.998	6.2	0.07	0.24

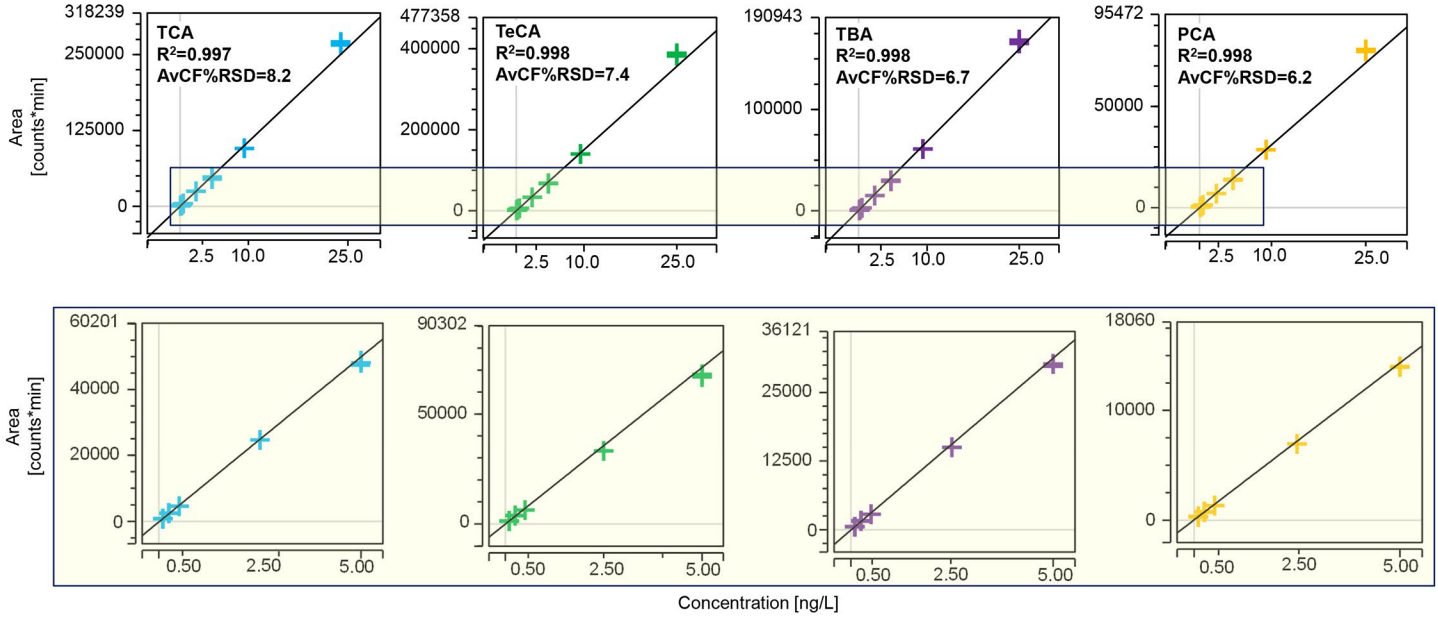

Figure 2. Calibration curves for haloanisoles. Coefficient of determination (R^2) and AvCF %RSD are annotated.

The method detection limits (MDLs) were determined by spiking n=9 Sangiovese red wine samples at 0.25 ng/L. MDLs were calculated considering the one-tailed Student's *t*-test value for the corresponding n-1 degrees of freedom at 99% confidence and multiplying it by the standard deviation of the replicated analyses. The limits of quantification (LOQs) were established by multiplying the standard deviation obtained in the MDL assessment by a factor of 10. Calculated MDLs and LOQs are reported in Table 4. As expected, compounds with a high boiling point, such as TBA and PCA in this case, showed higher MDLs and LOQs since lower amounts are extracted and enriched on the fiber as a result of i) their partitioning coefficient and ii) the incubation and extraction temperature (40 °C) adopted in the method.

Carry-over assessment

Carry-over was assessed by desorbing the PDMS Arrow fiber without performing any sample extraction (fiber blank) before and after the completion of the n=60 wine sample sequence. The TriPlus RSH Fiber Conditioning Station ensured effective heating and flushing of the fiber for reduced risk of carry-over. No traces of the investigated compounds were found in the fiber blank as demonstrated with the extracted ion chromatogram (XIC) comparison (quantification ions) of the blank run at the beginning and end of the 60-sample sequence, as reported in Figure 3.

Repeatability

Peak area repeatability was tested using n=9 Sangiovese wine samples spiked at 0.25 ng/L for all the target compounds. The TriPlus RSH Heatex Stirrer module, with its unique cycloidal stirring, ensured effective and reliable extraction and enrichment of analytes, allowing for highly repeatable peak areas with less than 10% RSD even at very low concentrations such as 0.25 ng/L, as reported in Figure 4.

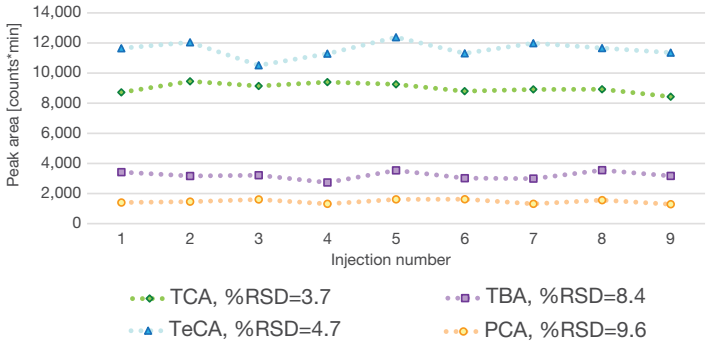

Figure 4. Precision of measurement as peak area %RSDs obtained for n=9 extractions of wine matrix spiked at 0.25 ng/L

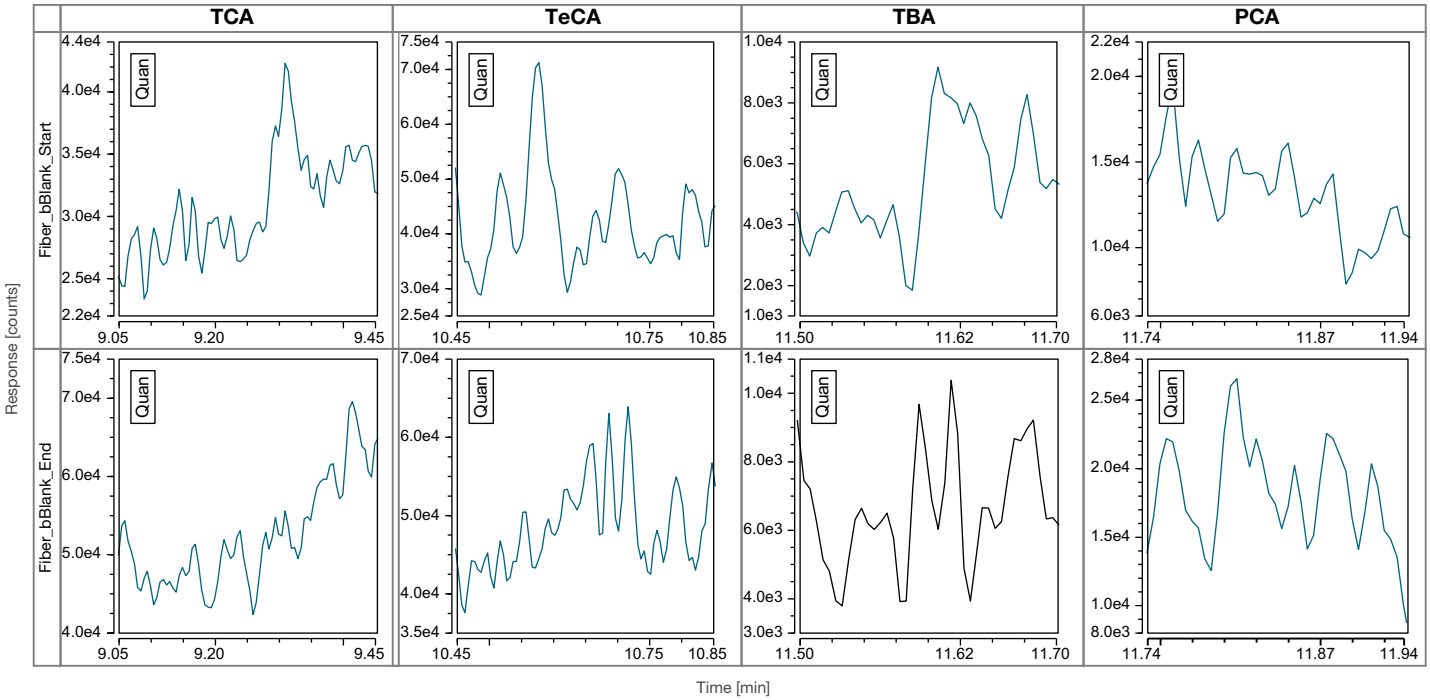

Figure 3. Fiber blank run at the beginning (top) and end (bottom) of a sequence containing a total of 60 samples

Quantitation of haloanisoles in wine samples

The system´s quantitative performance and stability were tested by running a 60-sample sequence containing a wine matrix-matched calibration curve (five calibration levels), red and white wine unknown samples, and quality controls (QCs) fully unattended over 36 hours. Some red and white wine samples were fortified at 2.5 ng/L and randomly analyzed across the sequence to evaluate the quantitative performance. QCs were spiked at 1.0 ng/L in model wine and injected every five samples to monitor the instrument performance.

The comparison of the XIC of a QC in model wine at 1.0 ng/L and the investigated wine matrices is shown in Figure 5. Small traces of TCA could be detected in the investigated wines as it can be present in the winery environment and is therefore very difficult to eliminate completely. The TCA amounts resulted < LOQ and its sensory threshold (2–5 ng/L). The amount of haloanisoles in the fortified samples and across the QCs was within ±25% of the spiked concentrations with i) ion ratios within 20% of the expected values, ii) RT standard deviation < 0.05 minutes, and iii) absolute peak area RSD < 10%, thus confirming reliable quantitation performance and good system stability can be obtained over time (Figure 6 and Appendix B). TBA was found to show the highest variability in terms of absolute peak area counts within the analyzed matrices showing lower values in Trebbiano wine. This can be due to the non-volatile wine matrix affecting the partitioning of the compounds between the matrix and the gas phase depending on the specific chemical properties of the analytes.[6]

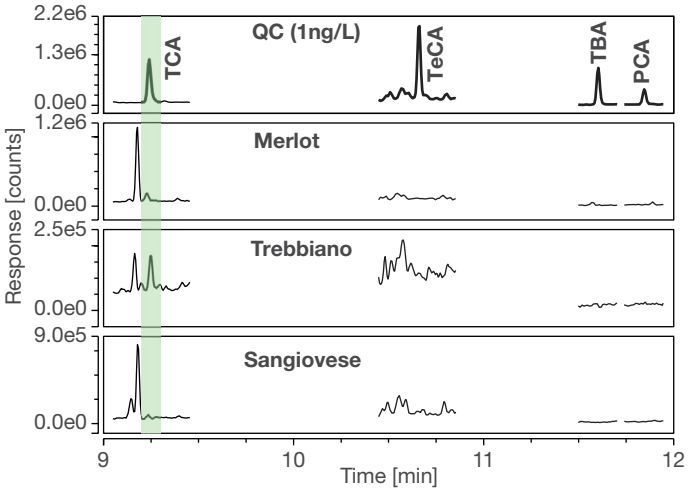

Figure 5. XIC comparison of QC in model wine at 1.0 ng/L and the investigated wine matrices

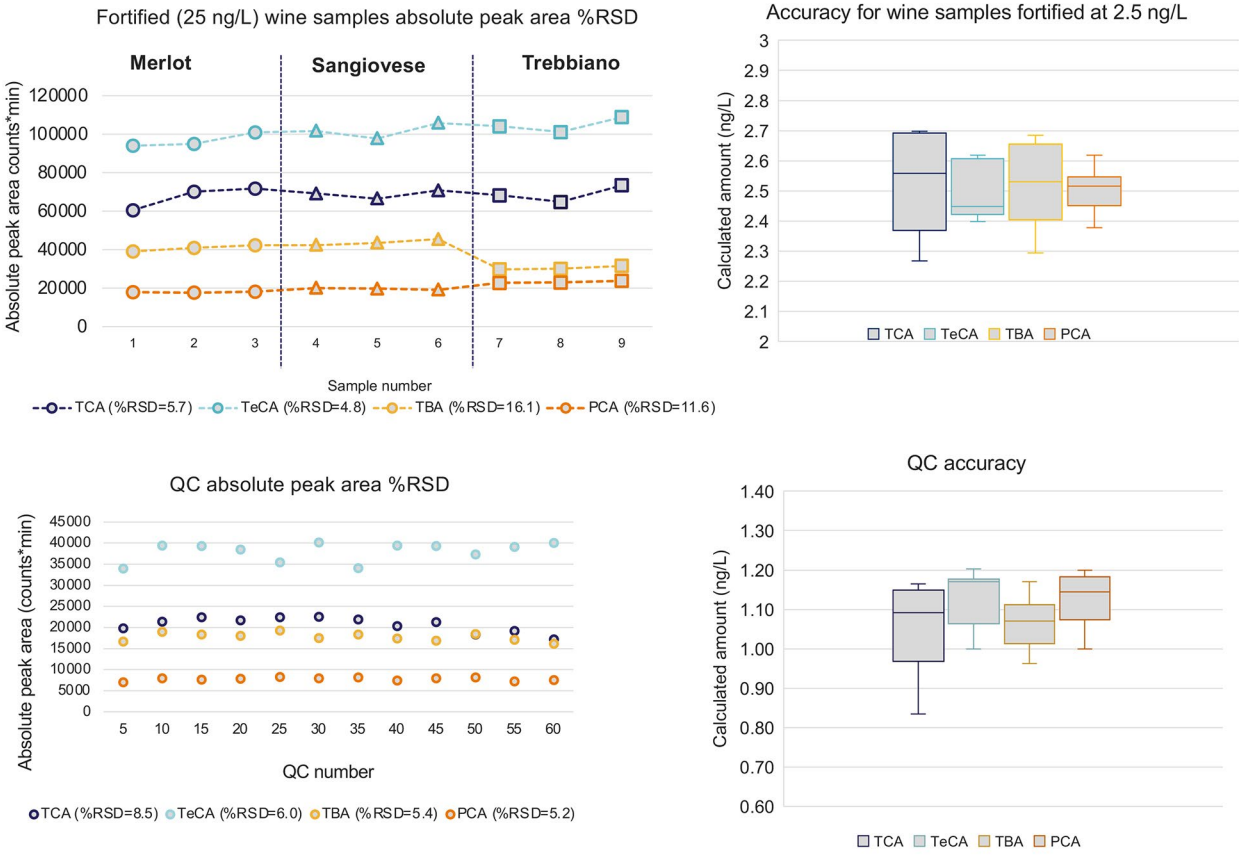

Figure 6. Robust performance as demonstrated by the analysis of both QC standards spiked at 1 ng/L and injected every five samples and fortified wine samples at 2.5 ng/L randomly extracted across the sequence. The calculated amounts were within 25% of the expected concentration with i) ion ratios within 20% the expected values, ii) RT standard deviation <0.05 minutes, and iii) absolute peak area RSD <16% for all compounds.

Conclusions

The results obtained demonstrate that the TSQ 9610 triple quadrupole GC-MS/MS system in combination with the TriPlus RSH SMART autosampler configured for HS-SPME Arrow sampling, allows for sensitive, fast, and robust analysis of haloanisoles in wine, making this configuration suitable for winery and analytical laboratories requiring fast and high-throughput assessment of wine quality.

- The minimal sample preparation, the fully automated workflow, and the short cycle time of HS-SPME Arrow sampling support laboratories facing high workload demand by unattended operations and reduced overall analysis time. All target compounds were separated in <15 min with consistent Gaussian peak shapes and baseline chromatographic resolution.
- Calculated recovery, evaluated for wine samples spiked at 0.25, 2.5, and 5.0 ng/L, was in the range of 90% to 105%.
- Linearity was assessed ranging from 0.1 to 25 ng/L and injecting every calibration level in duplicate. Average R^2 was ≥0.997 and AvCF % RSD <8.2 for the investigated compounds. Calculated MDLs were <0.08 ng/L and LOQs <0.25 ng/L, thus well below the analyte sensory thresholds.
- Red and white wine samples as well as fortified samples and QCs were analyzed across 36 hours running the instrument unattended. Traces of TCA (<LOQ) could be detected in the native samples as it can be present in the winery environment. The amount of haloanisoles in the fortified samples and across the QCs was within 25% of the spiked concentrations with i) ion ratios within 20% the expected values, ii) RT standard deviation <0.05 minutes, and iii) absolute peak area RSD <16% (reported in the Figure 6 caption), thus confirming that reliable quantitation performance and good system stability can be obtained over time.

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Appendix A

Table A1. List of standards and reagents, P/N, and supplier

Analytical standard / reagent	P/N	Supplier
2,4,6-trichloroanisole (TCA)	DRE-XA17714600ME	LGC Standards
2,3,4,6-tetrachloroanisole (TeCA)	DRE-X17333150HA	LGC Standards
Tribromoanisole (TBA)	DRE-L17664000IO	LGC Standards
Pentachloroanisole (PCA)	DRE-L15950000CY	LGC Standards
Tartaric acid (≥99.5%)	11377868	Fisher Scientific
Hydrochloric acid (37%)	10053023	Fisher Scientific
Sodium chloride (≥99%)	10127853	Fisher Scientific
Acetone (for residue analysis, 99.9%)	326570025	Fisher Scientific
Ethanol (absolute, 99,9%)	13268633	Fisher Scientific
HPLC-MS grade water	10777404	Fisher Scientific

Appendix B

Table B1. Absolute peak area counts and %RSD calculated for QC (1 ng/L) in model wine and fortified (2.5 ng/L) samples (M= Merlot, S=Sangiovese, T=Trebbiano) analyzed across a 60-sample sequence

	Absolute peak area (counts*min)				%RSD			
Target analyte	QC	M	S	T	QC	M	S	T
TCA	20790	67428	68800	68795	8.5	8.9	3.1	6.3
TeCA	38098	96603	101740	104700	6.0	3.8	4.0	3.7
TBA	17837	40803	43794	30467	5.4	4.0	3.6	3.2
PCA	7840	17897	19643	23147	5.2	1.5	2.5	2.4

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