APPLICATION NOTE 10752

Rapid smoke-taint analysis of wine with SPME-GC-MS/MS

Authors: Giulia Riccardino[1], Cristian Cojocariu[1], and Caroline Merrell[2]; [1]Thermo Fisher Scientific, Runcorn, UK, [2]Jackson Family Wines, Santa Rosa, California (USA)

Keywords: Smoke-tainted wine, wildfires, volatile phenols (VPs), gas chromatography-mass spectrometry, GC-MS/MS, triple quadrupole, TSQ 9000, ExtractaBrite, solid phase micro-extraction, SPME

Goal

The aim of this application note is to demonstrate the performance of the Thermo Scientific™ TSQ™ 9000 triple quadrupole mass spectrometer coupled to the solid phase micro extraction (SPME) technique for the determination of volatile phenols, VPs, as main markers of smoke impact in wines.

Introduction

Wildfires have been occurring more frequently in many parts of the world and create growing concerns for many important wine regions such as the USA, Australia, South Africa, Portugal, Chile, and Spain, among others. Such events lead to significant quantities of smoke being released into the atmosphere and transported over large distances.[1] When wildfires occur within the grape growing season, vineyards and grapes are exposed to smoke, and the resulting wines can have unwanted sensory characteristics. The resulting smoky, ashy, medical, and pharmaceutical tastes affect the wine quality and consequently the value on the market with significant financial losses.[2,3] Volatile phenols (VPs), such as guaiacol and 4-methylguaiacol, are mainly responsible for these smoky sensory characteristics and are therefore considered key markers of smoke-tainted wines. After permeating the grape skins these compounds can bind with the natural sugars in the berry tissues leading to the formation of conjugated glycosides. Conjugated glycosides are considered smoke taint precursors as they can further release free VPs during the vinification step or wine storage and maturation.[4] Therefore the determination of these compounds (in both free and conjugated forms) becomes critical to minimize the economic losses associated with producing smoke-tainted impacted wines.[2]

Currently there is no consensus on a recommended analytical method or a standardized protocol for the determination of free and conjugated VPs.[2] Most laboratories use gas-chromatography coupled to single quadrupole mass spectrometry (GC-MS) or triple quadrupole mass spectrometry (GC-MS/MS) as the preferred technology for this application. Headspace solid-phase microextraction (HS-SPME) has been shown to be a fast and effective sampling method when coupled to GC-MS analysis, and it has been used extensively for the determination of volatile compounds in wine.[2] The conjugated VPs can be analyzed either by LC-MS or by GC-MS/MS after acidic hydrolysis.[5] The LC-MS approach usually requires solid phase extraction (SPE) clean-up followed by a derivatization step because the direct analysis of phenolic compounds can suffer from ion suppression and poor sensitivity when electrospray ionization (ESI) is used.[5] Moreover, the limited availability of analytical and isotopically labeled standards for VP-glycosides poses further challenges when LC-MS is used.[5] GC–MS/MS coupled to HS-SPME following acidic-hydrolysis of the wine sample to release the VPs in their free form, represents a viable, cost-effective, and simple solution for the determination of free and glycoside VPs in wine.[5] In addition, the use of HS-SPME sampling has the advantage of providing artifact-free analyses, avoiding such effects when guaiacol and 4-methylguaiacol are injected as liquids at high temperatures.[6]

In this study, a fast and simplified analytical method was tested for the determination of free and conjugated VPs in finished wine. GC-MS/MS ensures appropriate selectivity and sensitivity for matrix samples using selected reaction monitoring (SRM). Additionally HS-SPME allows for fully automated sample extraction and pre-concentration of VPs.

Experimental

Instrumentation

In the experiments described here, a Thermo Scientific TSQ 9000 triple quadrupole mass spectrometer with Thermo Scientific™ NeverVent™ technology was coupled to a Thermo Scientific™ TRACE™ 1310 gas chromatograph equipped with a Thermo Scientific™ Instant Connect Split/Splitless (SSL) Injector and a Thermo Scientific™ TriPlus™ RSH autosampler configured for SPME. Chromatographic separation was achieved on a Thermo Scientific™ TraceGOLD™ TG-WAXMS capillary column, 30 m × 0.25 mm × 0.25 µm (P/N 26088-1420). The optimized extraction conditions and the triple coating phase of the DVB/CWR/PDMS fiber (P/N 36SP05T3) allowed for effective enrichment of the analytes of interest in only 10 minutes at 40 ˚C. The overlapping capability of the TriPlus RSH autosampler combined with a short GC oven ramp (15 min) ensured a short cycle time, enabling high sample throughput without compromising the chromatographic performance. Syringol was also included in the list of the target analytes but this compound required further method optimization due to its higher boiling point. In order to get adequate and effective extraction of syringol, the incubation temperature was increased to 80 ˚C and the extraction time was extended to 30 minutes. The results shown were acquired using the shortest method unless otherwise specified. Additional HS-SPME and GC-MS/MS parameters as well as a complete list of the target compounds are detailed in Table 1 and Table 2, respectively.

Table 1. HS-SPME and GC-MS/MS experimental conditions for the analysis of VPs. Syringol can be added to the target compounds but this requires a higher incubation temperature and a longer extraction time due to its lower volatility.

TRACE 1310 GC and TSQ 9000 triple quadrupole MS/MS parameters	
Inlet module and mode	SSL, Splitless
Liner (P/N 453A1335)	Direct straight liner
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)	15.00
TSQ 9000 triple quadrupole MS/MS parameters	
Ion source	Thermo Scientific™ ExtractaBrite™
Transfer line temperature (˚C)	250
Source temperature (˚C)	270
Ionization mode	EI
Electron energy (eV)	70
Acquisition mode	Selected reaction monitoring (SRM)
Chromatographic column	
TraceGOLD TG-WAXMS (P/N 26088-1420)	30 m × 0.25 mm × 0.25 µm

Table 1 (continued). HS-SPME and GC-MS/MS experimental conditions for the analysis of VPs. Syringol can be added to the target compounds but this requires a higher incubation temperature and a longer extraction time due to its lower volatility.

TriPlus RSH autosampler – SPME parameters		
Fiber	SPME DVB/CWR/PDMS (P/N 36SP05T3)	
Fiber outer diameter (gauge)	23	
Coating phase thickness (µm)	50/30	
Coating phase lenght (mm)	10	
Incubation time (min)	0.5	
	All VPs	**Syringol**
Incubation and extraction temperature (˚C)	40	80
Extraction time (min)	10	30
Analysis time (min)	20	
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	

Data acquisition, processing, and reporting

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

Standard and sample preparation

Guaiacol, 4-methylguaiacol, *o*-cresol, *p*-cresol, 4-ethylguaiacol, tartaric acid, hydrochloric acid (37%), and sodium chloride were purchased from Sigma-Aldrich. Syringol, eugenol, *m*-cresol, 4-ethylphenol, ethanol (absolute, 99.9%), sodium hydroxide (4 M), and HPLC-MS grade water were purchased from Fisher Scientific. Deuterated internal standards (guaiacol-d_3, 4-methylguaiacol-d_3 and *m*-cresol-d_7) were purchased from CDN Isotopes. The complete list of the P/Ns can be found in the Appendix.

Table 2. List of target VPs, retention times (RT, min), SRM precursor and product ions (*m/z*), and collision energies (eV)

Target analyte	RT (min)	Precursor ion (*m/z*)	Product ion (*m/z*)	Collision energy (eV)
Guaiacol-d_3	9.52	127	81	20
		127	109	12
Guaiacol	9.52	124	81	18
		124	109	10
4-methylguaiacol-d_3	9.96	126	70	10
		141	98	16
		141	126	10
4-methylguaiacol	9.96	123	67	10
		123	95	6
		138	123	10
o-cresol	10.13	79	77	10
		107	77	14
		108	107	12
4-ethylguaiacol	10.32	137	94	15
		137	122	10
		152	137	10
p-cresol	10.51	107	77	14
		108	77	24
		108	107	14
m-cresol-d_7	10.57	113	85	24
		115	85	24
		115	113	14
m-cresol	10.57	107	77	14
		108	77	24
		108	107	12
Eugenol	10.92	149	77	20
		164	131	10
		164	149	10
4-ethylphenol	10.92	107	51	25
		107	77	15
		122	107	10
Syringol	11.40	139	65	10
		154	65	15
		154	139	10

Standard preparation

Since VPs naturally occur in wine and they are also found in toasted oak barrels used in aging, a model wine (13% ethanol, 5g/L tartaric acid in 1,000 mL HPLC-MS grade water, final pH=3.5) was prepared and used to dilute both the pure and the internal standards.[6]

Internal standards were diluted using the model wine to a final concentration of 10 mg/L (ppm) and used to spike both the calibration solutions and the samples.

Mixed stock standard solution at 1,000 mg/L (ppm) was prepared and diluted using the model wine to obtain ten calibration solutions ranging from 0.1 to 100 µg/L (ppb). Each calibration solution (10 mL) was transferred into 20 mL headspace vials (P/N C4020-18, caps P/N 18-MSC-ST201) and spiked with 10 µL of ISTD mix. Each calibration level was prepared in duplicate.

NaCl (2 g) was added to the vials to improve the extraction efficiency of the target compounds.

Sample preparation for free VPs determination in wine samples

Four wine samples—Merlot (samples 1 and 2), Cabernet Sauvignon (sample 3), and Cabernet Franc (sample 4)—were provided from Jackson Family Wines (California, USA).

Calibration solutions and wine samples were used to assess recovery, method linearity, sensitivity, repeatability, quantitative performance, and system robustness.

Sample preparation for glycosylated VPs determination in wine samples

Although there are not official protocols for the assessment of conjugated phenols, various hydrolysis procedures have been reported in literature. Among these, acidic hydrolysis using hydrochloric acid (HCl, 37%) is by far the most simple and commonly used as reported in literature.[5] For assessment of conjugated phenols, wine samples (10 mL) were spiked with 10 µL ISTD mix, added with HCl (final pH=1.5) and incubated at 95 ˚C for 4 hours to allow the release of VPs in their free form. Samples were left to cool down at ambient temperature before spiking the vials with sodium hydroxide (NaOH, 4 M) to adjust the pH to 3.5. NaCl (2 g) was added before the analysis. Typically the validation of the hydrolytic procedure would be performed by spiking the samples with glycosylated VPs to evaluate the recovery and the efficiency. Due to some difficulties in outsourcing the glycosylated standards, repeatability of the hydrolytic procedure was evaluated by preparing duplicated aliquots of wine samples and assessing them over different days.

Results and discussion

Chromatography

Wine is a complex matrix containing a nonvolatile fraction, including polyphenolic compounds, proteins, and carbohydrates, and a volatile fraction, which includes hundreds of flavor and aroma compounds.[7] SPME headspace sampling reduces the complexity of the matrix with minimal sample preparation. SRM acquisition is highly selective and helps to discriminate between the target compounds and the matrix interferences. As an example, the total ion chromatogram (TIC) acquired in EI, full-scan mode (*m/z* 50–500), and the SRM acquisition of a wine sample spiked at 5 µg/L are shown in Figure 1. Chromatographic separation was achieved for the investigated compounds with the exception of eugenol and 4-ethylphenol (RT=10.92 min) that can be easily separated based on their characteristic ions. Gaussian peak shape was obtained for all target compounds with average asymmetry factor (A_s) of 1.0.

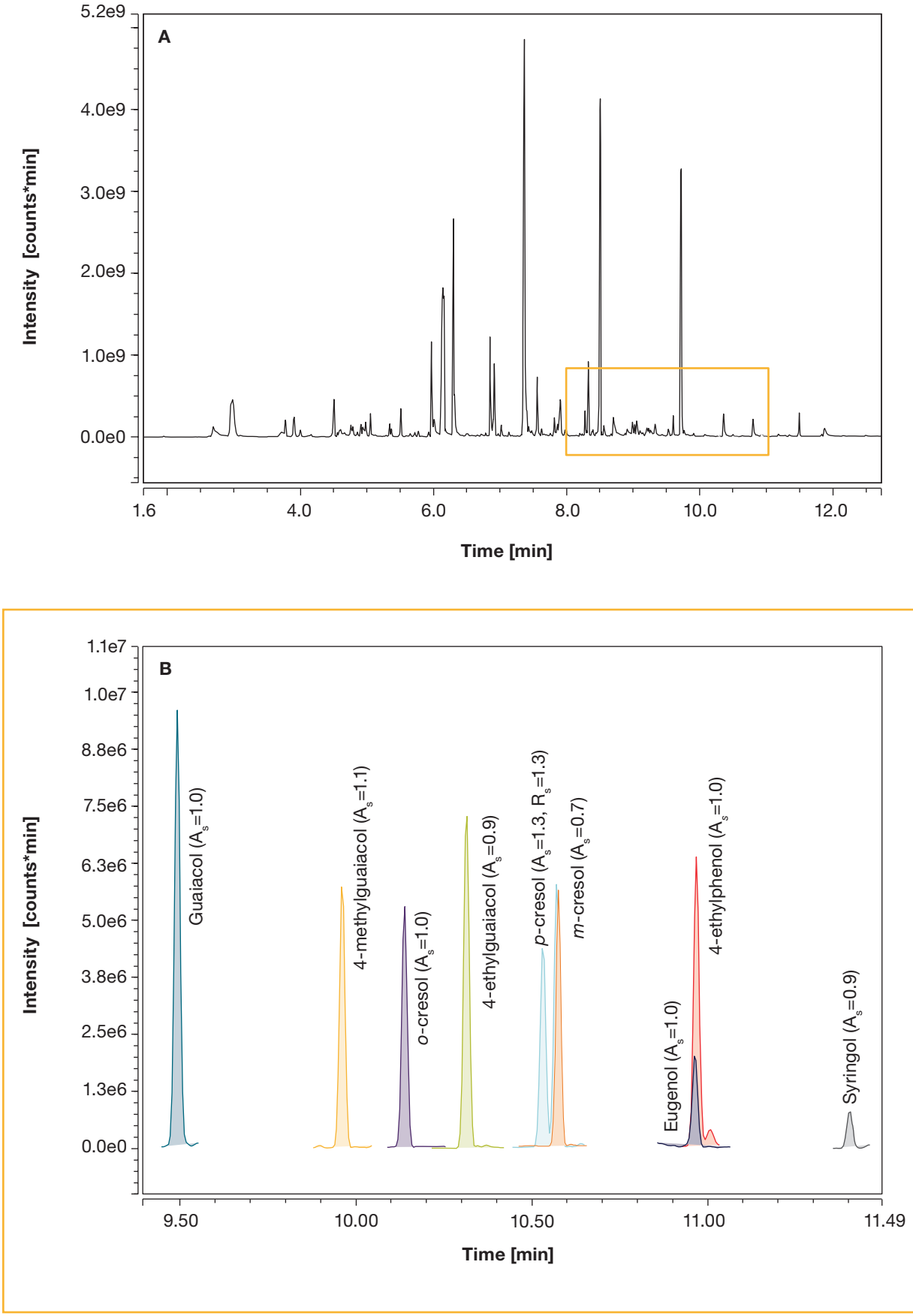

Figure 1. TIC (full scan: *m/z* 50–500, A-upper trace) and SRM acquisition (B-bottom trace) for a wine sample spiked at 5 µg/L. Chromatographic separation was achieved for all the investigated compounds with the exception of eugenol and 4-ethylphenol (RT=10.92 min) that can be easily separated based on their characteristic ions. A_s values and R_s for the critical pair *p,m*-cresol are annotated.

Analytes recovery

Recovery for free VPs was assessed by preparing three spiking solutions at 5, 25, and 50 µg/L in model wine. The spiking solutions were then used to spike three wine samples. Calculated recoveries were between 70 and 130% of the spiked concentration for the investigated analytes with the exception of *o*–cresol for which the recovery at 5 µg/L was 69% as reported in Table 3.

Table 3. Calculated recoveries of target compounds (%) from wine samples spiked at 5, 25 and 50 µg/L. Calculated recovery was between 70–130% with the exception of *o*–cresol for which the recovery at 5 µg/L was 69%.

Target analyte	RT (min)	Spiked concentration (µg/L)	Calculated concentration (µg/L)	Recovery (%)
Guaiacol	9.52	5	3.7	73
		25	19	75
		50	45	91
4-methylguaiacol	9.96	5	4.4	87
		25	24	95
		50	48	96
o-cresol	10.13	5	3.4	69
		25	19	77
		50	41	83
4-ethylguaiacol	10.32	5	4.2	85
		25	24	96
		50	44	87
p-cresol	10.51	5	3.9	78
		25	27	108
		50	50	99
m-cresol	10.57	5	3.7	74
		25	32	128
		50	49	98
Eugenol	10.92	5	4.8	96
		25	29	116
		50	48	96
4-ethylphenol	10.92	5	4.0	80
		25	22	87
		50	48	96

Linearity and method detection limit (MDL)

Calibration curves ranging from 0.10 to 100 µg/L (10 calibration levels) were used to assess method linearity and detection limits. Each calibration level was prepared and injected in duplicate. Linearity for syringol was evaluated by injecting six calibration standards ranging from 2.5 to 100 µg/L, applying the modified extraction conditions in Table 1. Adequate linearity was obtained with an average coefficient of determination (R^2) of 0.999 and an average calibration factor %RSD (AvCF %RSD) <10% for all investigated compounds (Table 3). Example calibration curves for guaiacol, 4-methylguaiacol, and syringol are reported in Figure 2.

The method detection limits were determined for all the target compounds by extracting n=10 standards at 0.25 µg/L, apart from syringol for which n=10 standards at 5 µg/L were used. MDLs were calculated considering the one-tailed Students t-test values for the corresponding n-1 degrees of freedom at 99% confidence and multiplying it for the standard deviation of the replicated analysis. Calculated MDLs are reported in Table 4. As expected, syringol showed a higher MDL (1.50 µg/L) since lower amounts are extracted from the fiber as a result of its partitioning coefficient, while for the most volatile VPs the MDL was <0.20 µg/L.

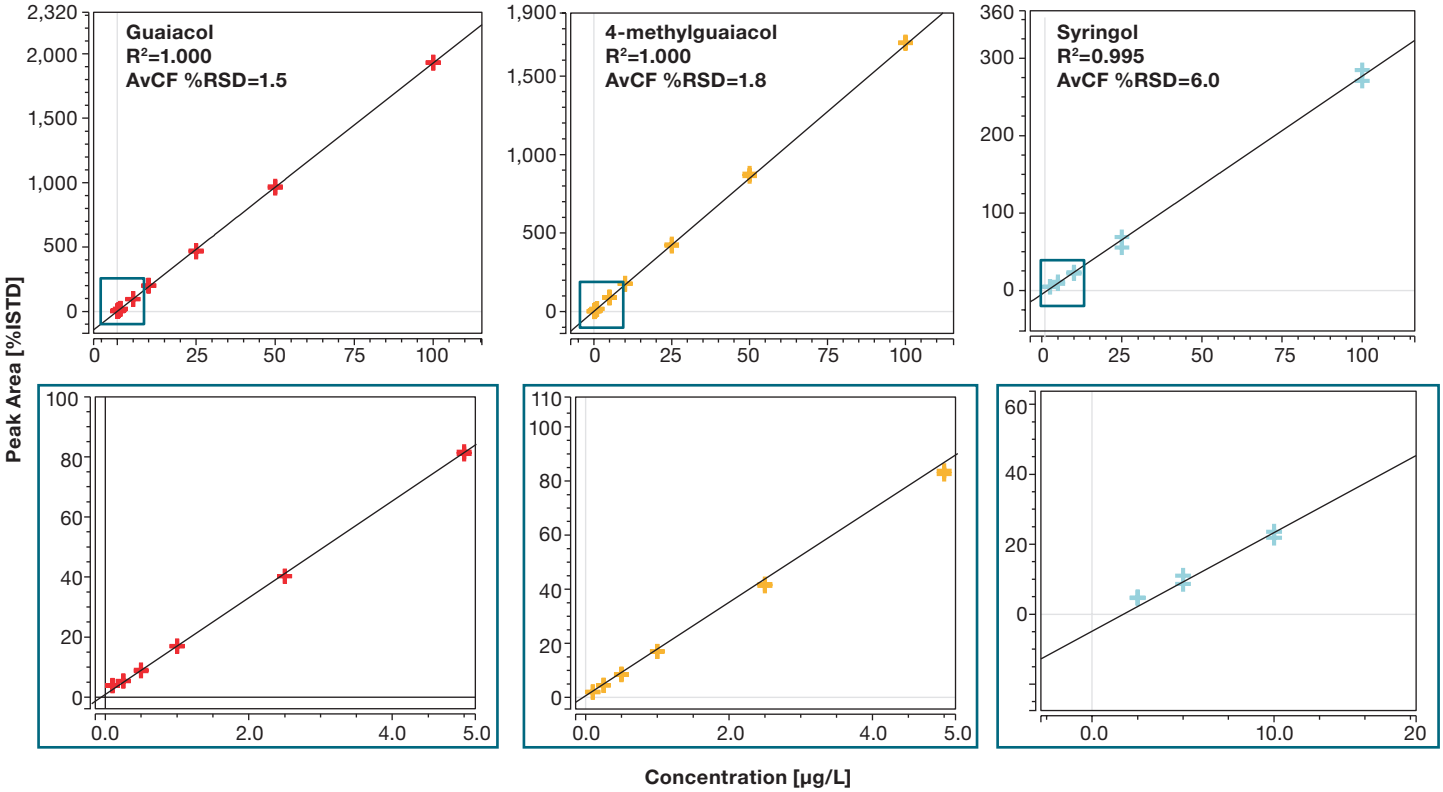

Figure 2. Examples of calibration curves for guaiacol, 4-methylguaiacol, and syringol. Each calibration level was injected in duplicate. Coefficient of determination (R^2) and AvCF %RSD are annotated.

Table 4. Coefficient of determination (R^2), average calibration factor (AvCF) %RSD, and calculated MDL (µg/L) for the target analytes. Adequate linearity was obtained with average correlation coefficient of 0.999 and AvCF %RSD <10%.

Target analyte	RT (min)	Coefficient of determination (R^2)	AvCF %RSD	Calculated MDL (µg/L)	Calculated carryover (%)	
					After calibration curve in model wine	After wine samples (n=2) spiked at 500 µg/L
Guaiacol	9.52	1.000	1.5	0.03	0.04	0.05
4-methylguaiacol	9.96	1.000	1.8	0.04	0.06	0.07
o-cresol	10.13	1.000	1.4	0.03	0.01	0.01
4-ethylguaiacol	10.32	0.999	2.1	0.03	0.05	0.08
p-cresol	10.51	0.999	3.0	0.16	0.01	0.03
m-cresol	10.57	0.999	2.5	0.05	0.01	0.04
Eugenol	10.92	0.998	5.7	0.04	0.06	0.05
4-ethylphenol	10.92	0.999	4.1	0.07	0.01	0.04
Syringol	11.40	0.995	6.0	1.50	0.01	0.14

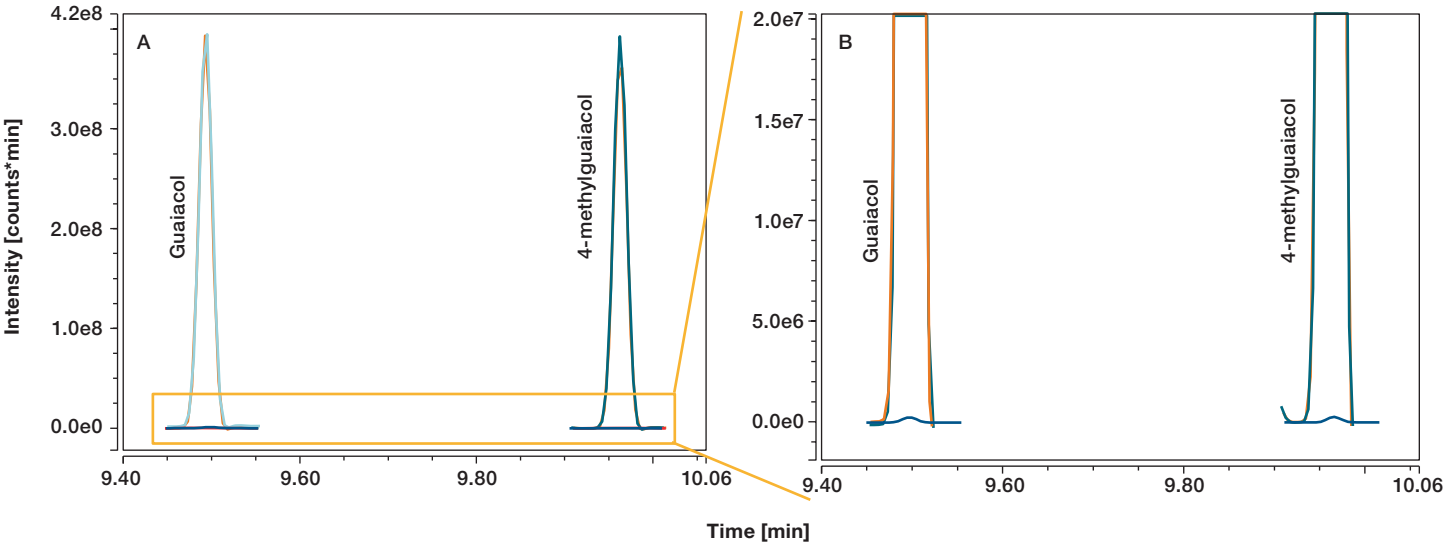

Figure 3. Example of carryover (blue trace) assessed running a fiber blank after extraction of wine samples (n=2) spiked at 500 µg/L, full scale (A), zoomed scale (B). Calculated carryover for both guaiacol and 4-methylguaiacol was <0.10%.

Carryover assessment

Target compounds carryover was assessed by desorbing the fiber without performing any sample extraction (fiber blank) before and after the calibration curve in model wine and before and after the extraction of two wine samples spiked at 500 µg/L. Calculated carryover resulted <0.10% for all target compounds in both model wine and real wine samples with the exception of syringol for which the carryover in wine samples was 0.14% as reported in Table 4. As an example, wine samples (n=2) spiked at 500 µg/L and the fiber blank after the extraction is reported for guaiacol and 4-methylguaiacol in Figure 3.

Repeatability

Peak area repeatability was tested using n=10 consecutive sample extractions at 0.25 µg/L for all the target compounds except for syringol for which the 5 µg/L level was used. The reliable extraction efficiency of the DVB/CWR/PDMS SPME fiber combined with the automated sampling process and the stability of the ExtractaBrite ion source allowed for %RSD <10 at very low concentrations, such as 0.25 µg/L as reported as an example in Figure 4. Peak area %RSD for syringol resulted 13%.

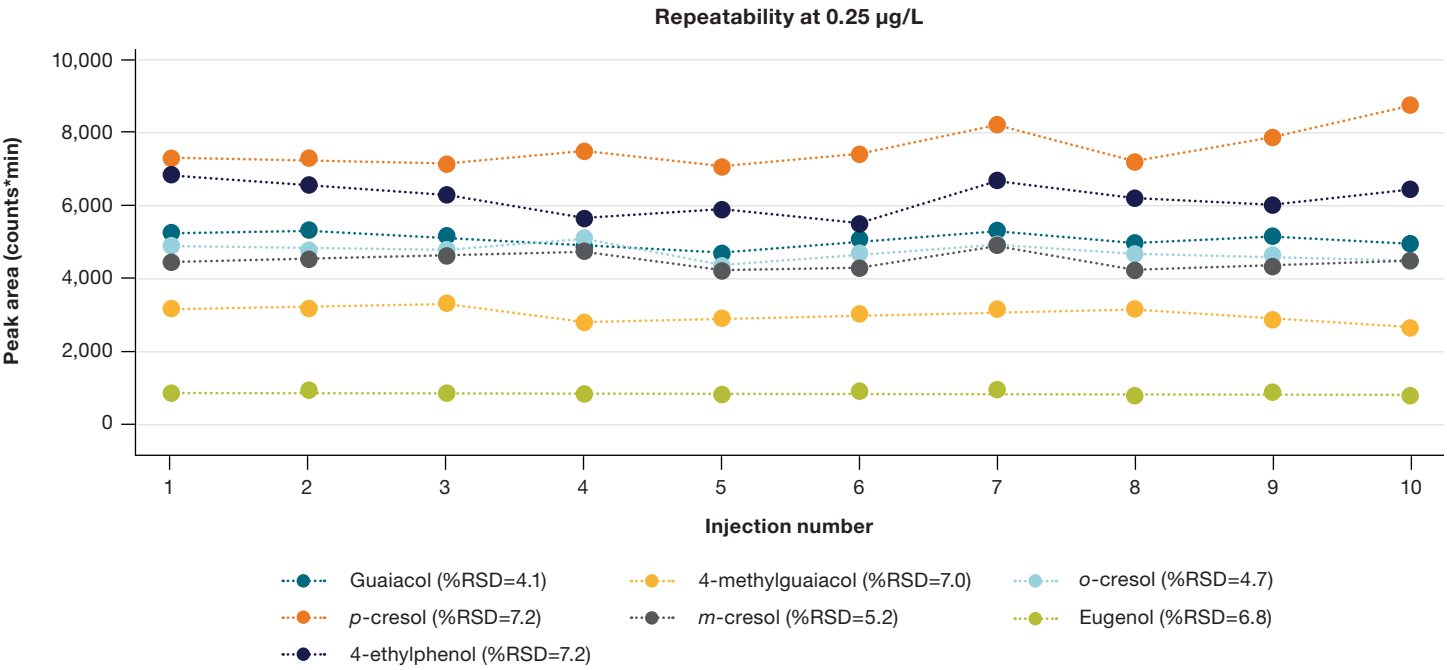

Figure 4. Precision of measurement as peak area %RSDs obtained for n=10 consecutive extractions at 0.25 µg/L were <10%.

Quantitation of free and conjugated VPs in wine samples

The TSQ 9000 SmartTune™ wizard was used to ensure consistency of mass spectrometer response for the quantitative assessment of free and conjugated VPs over time.

The method was tested with wine samples prepared in duplicate (one aliquot used to assess the free VPs and the second aliquot for the total VPs content). Samples, calibration standards, and QC standards were freshly prepared on the day of the analysis and analyzed over two different days (n=120 samples in total) using the conditions reported in Table 1. QCs consisted of spiked model wine samples (at 25 µg/L) that were injected every six samples to monitor the instrument performance. Common type 1, class A glass (borosilicate) vials for headspace analysis were used for acidic hydrolysis, although they can lead to low recoveries for several compounds including guaiacol and 4-ethylguaiacol when compared to PTFE reaction vessels. As reported in literature, this can depend on non-specific surface effects interfering with the assay.[5]

As expected, free VPs were detected in all samples as they naturally occur in wine. Samples 1 and 4 contain higher amounts of free guaiacol and 4-methylguaiacol as compared to samples 2 and 3 (Table 4). The amount of VPs increased after the hydrolysis in all the tested samples as a result of the glycosidic bond breakage. The total VP amounts calculated for samples 1 and 4 suggested a possible exposure of the grapes to bushfires with calculated values of guaiacol and 4-methylguaiacol greater than 10 µg/L and 5 µg/L, respectively. Calculated concentrations for free and total VPs were consistent across the replicated samples with average intra- and inter-day variations of ≤20% as reported in Table 5.

The method accuracy was confirmed with calculated amounts for spiked QCs within 20% of the spiked concentrations as reported in Figure 5.

Table 5. Average concentrations (µg/L), intra-and inter-day variations (%RSD) for free and total VPs in wine samples. Two aliquots of samples were prepared and analyzed for free and total VP content in two different days. Calculated amounts were consistent with average intra- and inter-day variations of 20%.

Sample	Form	Average amount (µg/L)								Average variation (%RSD)	
		Guaiacol	4-methylguaiol	*o*-cresol	4-ethylguaiacol	*p*-cresol	*m*-cresol	Eugenol	4-ethylphenol	Intra-day	Inter-day
1	Free	4.5	1.2	1.6	0.5	1.0	0.9	2.8	0.4	11	15
	Total	16.8	6.7	3.0	0.6	4.3	2.6	4.8	1.7	7	16
2	Free	1.7	0.6	0.7	0.5	0.7	0.4	2.6	0.3	16	19
	Total	6.1	2.2	1.5	0.6	2.1	1.2	4.9	1.7	10	20
3	Free	1.9	0.6	1.0	0.5	0.6	0.4	4.3	0.2	12	20
	Total	4.8	1.5	1.6	0.6	1.8	1.1	8.8	1.1	8	14
4	Free	6.0	2.4	1.5	0.7	1.8	0.9	4.8	0.6	7	14
	Total	13.7	5.6	2.2	0.8	3.4	1.9	8.5	2.2	7	14

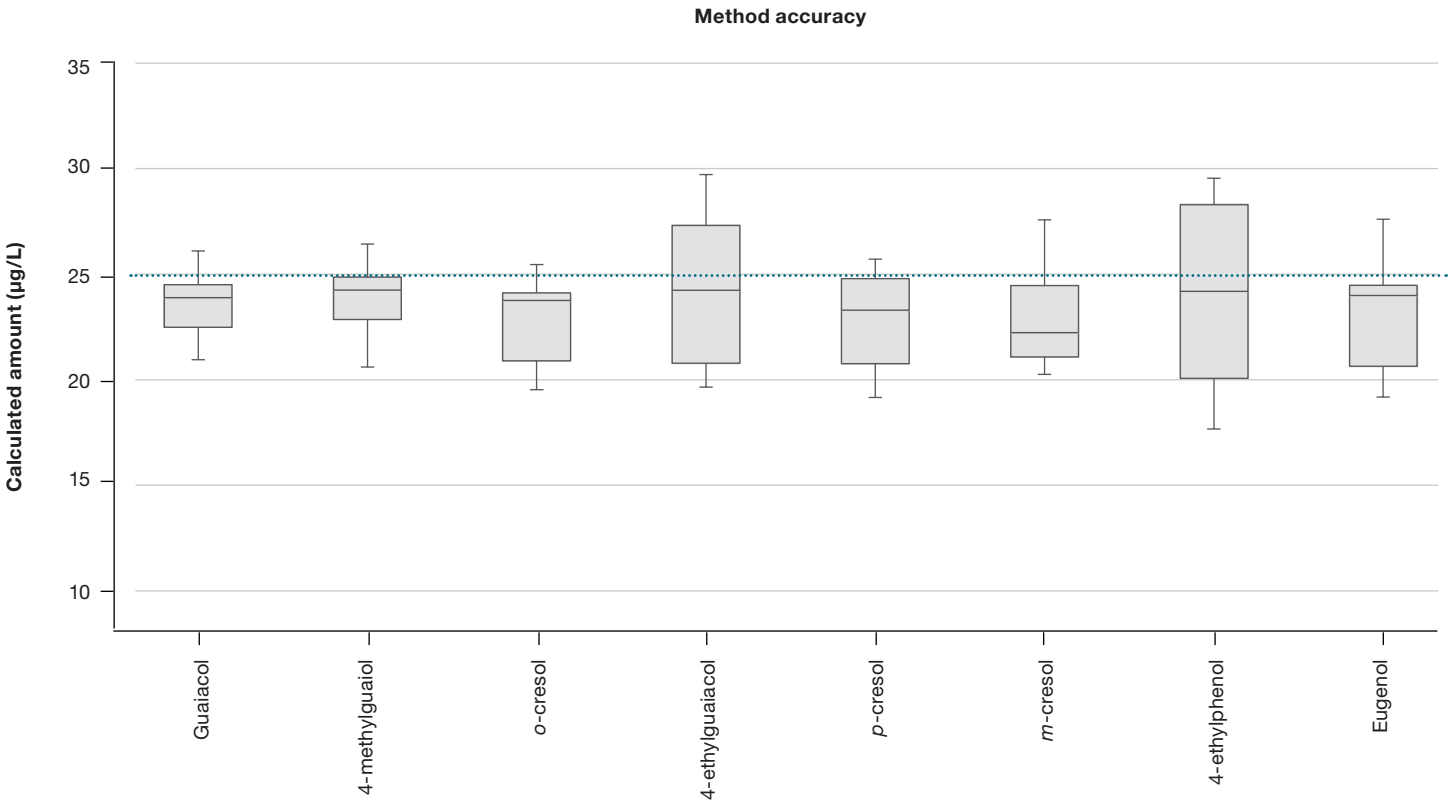

Figure 5. Robust performance as demonstrated by the analysis of QC standards spiked at 25 µg/L and injected every six samples. The calculated amounts were within 20% of the expected concentration for all compounds.

Conclusions

The automated workflow and the short cycle time support large testing laboratories to face high workload demand during the harvest months. All target compounds were separated in <12 min with consistent Gaussian peak shape and excellent chromatographic resolution, including the critical pair *p,m*-cresol.

- The scope of the analysis can be broadened by adding syringol to the target compounds but this requires different extraction conditions due to the chemical properties of this analyte, leading to longer extraction times therefore reducing the sample throughput.
- HS-SPME sampling almost removes the sample preparation step, reducing the overall analysis time. Moreover, it ensures fully automated sample extraction and pre-concentration in one single step.
- The TSQ 9000 SmartTune wizard and the overall system robustness ensured consistency of results with calculated amount for QC samples within ±20% of the spiked concentrations and average intra- and inter-day variations of sample measurements ≤20%.

The method developed in this HS-SPME-GC-MS/MS configuration was tested for typical performance parameters as well as for real wine samples:

- Linearity was assessed ranging from 0.1 to 100 µg/L and injecting every calibration level in duplicate. Average R^2 was >0.999 and AvCF %RSD <10% for all target compounds.
- Calculated MDL values were <0.20 µg/L for all target compounds with the exception of syringol for which the calculated MDL was 1.50 µg/L.
- Calculated recovery, evaluated for wine samples spiked at 5, 25, and 50 µg/L, was in the range of 69 to 130%.
- Four wine samples were assessed for free and total content of VPs. Acidic hydrolysis was used to break the glycosidic bond and release the phenol in their free form. Higher levels of free VPs were found after acidic hydrolysis, demonstrating that conjugated VPs represent a reservoir of VPs that can be released during the vinification and storage processes and contribute to negatively affect the wine taste.

thermo scientific

The results obtained demonstrate that the TSQ 9000 triple quadrupole GC-MS/MS system in combination with the TriPlus RSH autosampler configured for HS-SPME sampling allows for fast and robust analysis of free and glycosidically bound VPs in wine, making this configuration suitable for winery laboratories and wine analytical labs requiring fast and high-throughput testing for wine quality assessment.

References

1. Krstic, M.P.; Johnson, D.L.; Herderich, M.J. Review of smoke taint in wine: smoke-derived VPs and their glycosidic metabolites in grapes and vines as biomarkers for smoke exposure and their role in the sensory perception of smoke taint, *Australian Journal of Grape and Wine Research* **2015**, *21,* 537–553.
2. https://www.awri.com.au/industry_support/winemaking_resources/smoke-taint/
3. https://www.abc.net.au/news/2020-02-20/bushfire-smoke-wipes-out-2020-vintage-at-canberra-wineries/11980862
4. Liu, Z.; Ezernieks, V.; Reddy, P.; Elkins, A.; Krill, C.; Murphy, K.; Rochfort, S.; Spangenberg, G. A simple GC-MS/MS method for determination of smoke taint-related VPs in grapes, *Metabolites* **2020**, *10,* 294.
5. Noestheden, M.; Thiessen, K.; Dennis, E.G.; Tiet, B.; Zandberg, W.F. Quantitating organoleptic VPs in smoke-exposed Vitis vinifera berries, *Journal of Agricultural and Food Chemistry* **2017**, *65,* 8418–8425.
6. Pollnitz, A.P.; Pardon, K.H.; Sykes, M.; Sefton, M.A. The effects of sample preparation and gas chromatograph injection techniques on the accuracy of measuring guaiacol, 4-methylguaiacol and other volatile oak compounds in oak extracts by stable isotope dilution analyses, *Journal of Agricultural and Food Chemistry* **2004**, *52,* 3244-3252.
7. Villamor, R.; Ross, C.F. Wine matrix compounds affect perception of wine aromas, *Annual Review of Food Science and Technology* **2013**, *4,* 1–20.

Appendix

Standard / reagent	P/N	Supplier
Guaiacol	G5502-100G	Sigma-Aldrich
4-methylguaiacol	41340-1mL	Sigma-Aldrich
o-cresol	C85700-100g	Sigma-Aldrich
4-ethylguaiacol	39774-1 mL	Sigma-Aldrich
p-cresol	61030-25g-F	Sigma-Aldrich
Tartaric acid	251380-100g	Sigma-Aldrich
Hydrochloric acid (37%)	320331-500mL	Sigma-Aldrich
Sodium chloride	S9888-1kg	Sigma-Aldrich
m-cresol	10367523	Fisher Scientific
Eugenol	11406897	Fisher Scientific
4-ethylphenol	10306560	Fisher Scientific
Syringol	11411307	Fisher Scientific
Ethanol (absolute, 99,9%)	13268633	Fisher Scientific
Sodium Hydroxide (4 M)	15614920	Fisher Scientific
HPLC-MS grade water	10777404	Fisher Scientific
Guaiacol-d_3	D-5968	CDN Isotopes
4-methylguaiacol-d_3	D-6963	CDN Isotopes
m-cresol-d_7	D-5637	CDN Isotopes

Find out more at **thermofisher.com**

ThermoFisher SCIENTIFIC