Research Note
Investigation of Thiol Levels in Young Commercial South African Sauvignon Blanc and Chenin Blanc Wines Using Propiolate Derivatization and GC-MS/MS

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Submitted for publication: October 2017
Accepted for publication: August 2018

Key words: Thiols, Sauvignon Blanc, Chenin Blanc, ethyl propiolate, GC-MS/MS

In this work, the ethyl propiolate method for analysing thiols in white wine by GC-MS, originally proposed by Herbst-Johnstone *et al.*) (2013), has been adapted to GC-MS/MS and has been validated. The method performance has shown improvement in terms of sensitivity (limit of detection, LOD) and of the number of compounds measured. In addition to 3-mercaptohexanol (3MH), 3-mercaptohexyl acetate (3MHA), and 4-mercapto-4-methylpentan-2-one (4MMP), the adapted method can also measure 2-furanmethanethiol (FMT) and makes use of a commercially-available internal standard (IS), 4-methoxy-2-methyl-2-butanethiol (4M2M2B, IS). The proposed method was applied to determine thiol levels in young commercial South African Sauvignon and Chenin Blanc wines. The samples (n=20 for each cultivar) were chosen according to a high frequency of the typical descriptors associated with this class of impact compounds. 3MH was found at 178-904 ng/L and 99-1124 ng/L, and 3MHA at 23-151 ng/L and 5-253 ng/L in Sauvignon and Chenin Blanc respectively. 4MMP was present in Sauvignon Blanc in concentrations up to 21.9 ng/L, but in none of the Chenin Blanc samples.

INTRODUCTION

Thiols are recognized as impact compounds with typical sensory attributes in wine, such as 'passionfruit', 'grapefruit', 'tomato leaf", and 'gooseberry', depending on the nature of the compound and the level at which they are present (Coetzee & du Toit, 2012). The challenge in measuring thiols in wine comes from both their reactivity (Danilewicz *et al.*, 2008)sulfur dioxide, and 4-methylcatechol (4-MeC and the ultra-trace concentration levels (Coetzee & du Toit, 2012; van Wyngaard, 2013; Wilson, 2017).

Until now, most of the methods for thiol determination have focused on the protection of these compounds against oxidation during the sample preparation steps. In these cases, the sample preparation had as purpose the isolation of the thiols from the interfering matrix and, at the same time, the concentration to levels that would allow the thiol determination by using an appropriate detection, usually MS. Since thiols give low ionization and therefore a weak signal in MS, the signal had to be boosted either by increasing the concentration in the injected extract (Tominaga *et al.*, 1998, 2000), or by a combination between concentrating and derivatizing the thiols before the instrumental analysis (Piano *et al.*, 2015). Another approach also made use of derivatization, but that was in the initial stages of the sample preparation (Herbst-Johnstone *et al.*, 2013; Capone *et al.*, 2015; Musumeci *et al.*, 2015; Vichi *et al.*, 2015). The methods using ethyl propiolate (Herbst-Johnstone *et al.*, 2013) and pentafluorobenzyl bromide (Musumeci *et al.*, 2015) as derivatization reagents have the advantage that the thiol derivatives formed in the initial steps of the sample preparation are resistant against oxidation as well as thermally stable. Firstly, the resistance against oxidation allows for easier manipulation of samples during the preparation steps, and secondly, the thermal stability allows determination of thiols by GC-MS. In the other two cases, 4,4′-dithiodipyridine (Capone *et al.*, 2015) and ebselen (Vichi *et al.*, 2015) were used as derivatization reagents which acted as stabilising agents against oxidation, but the chromatographic determination was done by LC-MS. Additionally, all the derivatives previously mentioned gave an acceptable signal in MS, for an approximate 2500-fold concentration from the initial sample.

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Acknowledgments: The authors would like to thank Winetech and THRIP for financial support, and IGWS for technical support

In this work, the ethyl propiolate method has been adapted to GC-MS/MS with an additional thiol (2-furanmethanethiol, FMT) included in the analysis, and has also been validated. The addition of FMT was done to prove the selectivity of the method, to which new compounds can be added without any detrimental effect to the initial analytes of choice. FMT was previously reported in some white wines (Tominaga & Dubourdieu, 2006), but there was no information available on South African wines. Since the presence and relevance of thiols has already been established for a number of years in South African Sauvignon Blanc wines (van Wyngaard, 2013; Piano *et al.*, 2015) and recently demonstrated in South African Chenin Blanc wines (Wilson, 2017), the proposed method was applied to determine thiol levels in young commercial South African wines (2016 vintage) from these two cultivars. To our knowledge, this is the first time that these levels are reported for young South African wines. Additionally, thiols were never determined using a combination of ethyl propiolate as derivatization reagent and GC-MS/MS.

MATERIALS AND METHODS

Sample preparation

The sample preparation is based on the method proposed by Herbst-Johnstone *et al.* (2013). Briefly, 50 mL of wine was combined with 500 µL 2 mM butylated hydroxyanisole (BHA, Sigma-Aldrich), 50 µL concentrated 4-methoxy-2-methyl-2-butanethiol (4MM2B, internal standard, Sigma-Aldrich), and 500 µL 250 mM ethyl propiolate (ETP, Sigma-Aldrich). The mixture was stirred for 5 minutes at 500 rpm, after which the pH was adjusted to 10±0.05 with NaOH (Sigma-Aldrich), and the mixture was again stirred for 10 minutes at 500 rpm. The precipitate formed was removed by centrifugation for 10 minutes at 6000 rpm, and the supernatant was transferred into a beaker for the SPE procedure.

SPE cartridges (Supelclean ENVI-18 SPE, Supelco) were conditioned with 10 mL methanol, followed by 10 mL Milli-Q water (Millipore). The wine sample was loaded, then the cartridge was washed with 5 mL Milli-Q water and dried for 20 minutes under vacuum. The analytes were eluted with 10 mL DCM (Sigma-Aldrich) and the eluate was then dried with anhydrous Na_2SO_4 (Sigma-Aldrich) to remove water traces. The DCM extract was evaporated under a gentle stream of N_2 down to approximately 100 µL, then injected into the GC-MS/MS.

Instrumental analyses

The instrumental analyses were on a Thermo Scientific TRACE 1300 gas chromatograph coupled to a Thermo Scientific TSQ 8000 triple quadrupole Mass Spectrometer detector (MSD). Chromatographic separation was performed on a polar Zebron ZB-FFAP capillary column (30 m x 0.25 mm x 0.25 µm, Phenomenex). The other instrumental parameters were as proposed by Herbst-Johnstone *et al*. (2013), with the following changes: the initial oven temperature was 60°C, held for 1 minute, then ramped up to 100°C at 25°C/min, held for 2 minutes, and finally ramped up to 250°C at 12°C/min, held for 5 minutes. Sample injection was done on the GC injection port with temperature maintained at 240°C operated in splitless mode with the split flow set at 50 mL/min for 2 minutes. Gas saver was activated for 5 minutes at 20 mL/min. Helium was used at 1.2mL/min as carrier gas. Both the transfer line and ion source temperatures were set at 250°C. Emission current of 75 µA and Argon was used as collision gas.

Detection was done in selected reaction monitoring (SRM) mode. The transitions monitored for each compound are presented in Table 1. For each compound, two transitions were monitored and most abundant daughter ion was used for quantification.

Method performance parameters

Selectivity

The selectivity of the chromatographic method was evaluated in model wine and white wine. Each thiol derivative was injected and the retention time and SRM transitions were recorded for future peak identification and quantitation (Table 1). In addition to retention times (RTs) the transitions monitored for each derivative ensured additional selectivity for the method.

TABLE 1
Thiols determined by the ETP-derivatization method, corresponding derivatives, their retention times and MS/MS transitions used for identification and quantitation.

Compounds	*Derivatives*	*Derivatives' Mw (g/mol)*	*Retention time (min)*	*MS-MS transitions (m/z)*
3-mercaptohexanol (3MH)	3MH-ETP	232	19.6	131.8 → 58.1 131.8 → 86
3-mercaptohexyl acetate (3MHA)	3MHA-ETP	274	17.9	229.1 → 83.1 84.8 → 57
4-mercapto-4-methylpentan-2-one (4MMP)	4MMP-ETP	230	16.5	132 → 86 132 → 58
2-furanmethanethiol (FMT)	FMT-ETP	212	16.7	212 → 179.1 130.9 → 103
4-Methoxy-2-methyl-2-butanethiol (4MM2B, IS)	4MM2B-ETP	232	15.0	200 → 126 132 → 86

 DOI: http://dx.doi.org/10.21548/39-2-2683

Linearity

The calibration ranges for the various thiols are presented in Table 2. Linearity was evaluated in model wine and white wine using the internal standard method. LOD values were calculated for a S/N of 3.

Precision

Precision was evaluated for the entire procedure (derivatization, extraction, and instrumental analysis) through repeatability tests over two days at two different concentration levels (medium-low and medium-high) for each thiol in both model and white wine. The results were expressed as % relative standard deviation (%RSD).

Accuracy

Accuracy was evaluated through recovery tests at the same two levels as precision. Concentration values obtained from model wine (non-interfering matrix, theoretical value) were compared to the ones from white wine (interfering matrix, practical value), and the recovery expressed as %.

Samples

The samples (n=20 for each cultivar) were chosen according to a high frequency of the typical descriptors associated with thiols. The descriptors for each wine were sourced from the bottle labels and tasting notes. All samples were analysed within two months after bottling (vintage 2016). Two of the Chenin Blanc samples (WMC 139 and WMC 174) were tank samples, while all other wines were commercially available.

RESULTS AND DISCUSSION

Method performance

Method performance in terms of linearity, LOD (ng/L), repeatability, and accuracy is presented in Table 2. All calibrations were linear in both model wine and white wine, with R^2 values higher than 0.99 in all cases. LOD was found better than the previously reported values for the same derivatization method in both model wine and white wine (Herbst-Johnstone *et al.*, 2013). This is most probably due to the higher sensitivity of the detection used in this case (MS/MS) compared to the literature cited (MS). LOD values are not always better than the odour threshold (OT) of the compounds, but the OT levels span an almost 10-fold range (0.8 vs. 60 ng/L). The repeatability and accuracy of the method are within acceptable limits.

Thiol levels in young South African wines

The results are presented in Table 2. As can be observed, young Chenin Blanc and Sauvignon Blanc wines had similar levels of 3MH and 3MHA. 3MH was found at 178-904 ng/L and 99-1124 ng/L, and 3MHA at 23-151 ng/L and 5-253 ng/L in Sauvignon and Chenin Blanc respectively. 3MH and 3MHA levels in South African Sauvignon Blanc wines were in general in line or lower than those reported for New Zealand Sauvignon Blanc wines (Herbst-Johnstone *et al.*, 2013), where the same derivatization method was employed. The main difference noted between the wines made from the two cultivars was in the presence of 4MMP in Sauvignon Blanc (not detected, n.d. – 21.9 ng/L), while no 4MMP was found in any of the Chenin Blanc samples analysed. The previous method used for the analysis of South African Chenin Blanc wines could not measure 4MMP (Piano *et al.*, 2015); therefore, even if the levels of 3MH and 3MHA in Chenin Blanc were already known to be similar to those in Sauvignon Blanc (van Wyngaard, 2013; Wilson, 2017), the absence of 4MMP in Chenin Blanc is a new finding. Even though the method can measure FMT, this thiol was not found in the samples analysed. These results can contribute to the advancement of the knowledge we have on the presence and levels of thiols in South African Chenin Blanc wines.

CONCLUSIONS

The proposed method was proven suitable for the analysis of thiols of interest in white wine. Using MS/MS detection improved LOD levels compared to the previously published ethyl propiolate GC-MS method. The use of a commercially available, non-deuterated IS was demonstrated to be acceptable. FMT was added to the list of thiols that can be measured using this method. Levels of 3MH and 3MHA in young South African Sauvignon Blanc and Chenin Blanc

TABLE 2
Figures of merit for the method parameters.

Compound	*matrix*	*OT (ng/L)* [1]	*Calibration range (ng/L)*	*R^2*	*LOD (ng/L)*	*LOD* (ng/L)*	*Repeatability** (%)*	*Accuracy (%)*
3MH	*MW*	60	100-4000	0.9994	2.1	9	11.3	104
	WW			0.9921	1	194.6	10.5	
3MHA	*MW*	4.2	50-1500	0.9943	3.8	1.5	12.1	104
	WW			0.9997	25	120.9	4.8	
4MMP	*MW*	0.8	10-300	0.9951	0.5	1.7	10.8	96.7
	WW			0.9992	10	24.5	12.5	
FMT	*MW*	50	5-80	0.9907	0.6	--	7.4	112
	WW			0.9976	2.5	--	8.0	

[1](Coetzee & du Toit, 2012)**previously reported, using same sample preparation, and GC-MS analysis (Herbst-Johnstone et al. 2013); **of entire procedure, including sample preparation and instrumental measurement*

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TABLE 3
Thiol concentrations (ng/L) for the young Chenin Blanc and Sauvignon Blanc samples.

	Chenin Blanc Concentration (ng/L)				Sauvignon Blanc Concentration (ng/L)		
Winery code	4MMP	3MHA	3MH	Winery code	4MMP	3MHA	3MH
BCW	n.d.	242	1124	BCW	3.5	132	897
BOC	n.d.	63	419	BOC	7.1	82	484
DLC	n.d.	40	298	DLC	0.7	31	322
VLN	n.d.	253	1091	VLN	n.d.	43	331
CDB	n.d.	144	541	CDB	7.3	146	767
KZC	n.d.	186	981	KZC	9.5	70	369
RBK	n.d.	64	280	RBK	2.6	62	196
DTK	n.d.	5	99	DTK	3.5	48	428
FRV	n.d.	29	182	FRV	21.9	39	213
BDB	n.d.	144	656	ANR	5.9	23	197
BSE	n.d.	94	514	BMT	6.4	151	860
DWC	n.d.	199	634	DMD	3.5	103	600
KCC	n.d.	35	260	DPC	3.3	45	286
NUY	n.d.	94	835	DVH	6.4	61	490
PDB	n.d.	53	328	FSC	3.5	72	397
SGH	n.d.	171	721	NE	9.4	41	245
SMS	n.d.	106	292	NJB	7.7	34	178
WLW	n.d.	78	412	NTH	3.9	37	495
WMC 139	n.d.	139	429	SNB	10.4	99	904
WMC 174	n.d.	171	536	TT	0.4	78	541

wines were found to be in the same range. Furthermore, 4MMP was found in Sauvignon Blanc up to 21.9 ng/L, but in none of the Chenin Blanc samples. More research is required to ascertain if 4MMP is absent in South African Chenin Blanc wines

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