Thiol levels in young commercial South African Sauvignon Blanc and Chenin Blanc wines using propiolate derivatization and GC-MS/MS

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Background

Thiols are recognized as character impact compounds, with typical sensory attributes such as ‘passionfruit’, ‘grapefruit’, ‘tomato leaf’, and ‘gooseberry’, depending on the level at which they are present. The challenge in measuring thiols in wine comes from both their reactivity and the ultra-trace levels at which they are present. The method proposed by Herbst-Jonstone *et al*. (2013) using ethyl propiolate as derivatization reagent has the advantages that the thiol derivatives are formed in the initial steps of the sample preparation, are resistant against oxidation and thermally stable, which allows their determination by GC-MS. In this work, the propiolate method has been adapted to GC-MS/MS and validated. The method performance has shown improvement in terms of sensitivity (LOD/LOQ) and of the number of compounds measured. The use of a commercially-available, non-deuterated IS was demonstrated to be acceptable.

Procedure

✓50 mL wine
✓500 µL BHA 2 mM
✓50 µL IS (final conc. 250 ng/L)
✓500 µL ETP 250 mM

→ - *Stir 5 min*
- *Adjust pH to 10±0.05*
- *Mix 10 min*
- *Centrifuge*

→ ✓supernatant →

Supelclean ENVI-18 SPE
- Condition: meOH, water
- Load sample
- Wash: water
✓ Elute: DCM

→ - *Dry sample (Na_2SO_4 anh.)*
- *Concentrate under N_2*

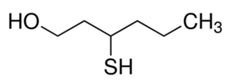
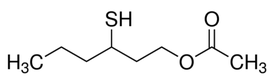
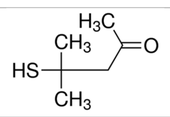
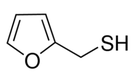
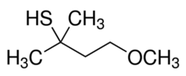
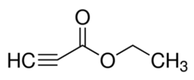
→ 

Thermo Scientific TSQ 8000 Evo triple quadrupole GC-MS system

Thermo TSQ 8000 PTV
Column: Agilent HP-INNOWax
30 m x 0.25 mm x 0.25 µm

Method Parameters

Thiol structures, molecular masses and transitions for the derivatives are presented in the Table below. The transitions monitored for each derivative (parent ions → daughter ions) ensure additional selectivity and sensitivity for the method.

Compounds	***Structures***	***Derivatives’ Mw (g/mol)***	***MS-MS transitions (m/z)***
3-mercaptohexanol (3MH)		3MH-ETP 232	131.8 → 58.1 131.8 → 86
3-mercaptohexyl acetate (3MHA)		3MHA-ETP 274	229.1 → 83.1 84.8 → 57
4-mercapto-4-methylpentan-2-one (4MMP)		4MMP-ETP 230	132 → 86 132 → 58
2-furanmethanethiol (FMT)		FMT-ETP 212	212 → 179.1 130.9 → 103
4-Methoxy-2-methyl-2-butanethiol (4MM2B, IS)		4MM2B-ETP 232	200 → 126 132 → 86
Ethyl propiolate (ETP, reagent)		--	--

Method Performance

Method performance in terms of LOD (ng/L), repeatability, and accuracy are presented below. LOD is in all cases better than the previously reported values for the same derivatization method in both model wine and white wine. 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 are within acceptable limits.

Compound – matrix	***OT (ng/L)***	***LOD (ng/L)***	***LOD* (ng/L)***	***Repeatability** (%)***	***Accuracy*** (%)***
3MH MW	60	2.1	9	11.3	104
WW		1	194.6	10.5	
3MHA MW	4.2	3.8	1.5	12.1	104
WW		25	120.9	4.8	
4MMP MW	0.8	0.5	1.7	10.8	96.7
WW		10	24.5	12.5	
FMT MW	50	0.6	--	7.4	112
WW		2.5	--	8.0	

**previously reported, using same sample preparation, and GC-MS analysis (Herbst-Johnstone et al. 2013) ; **of entire procedure, including sample preparation and instrumental measurement; ***white wine vs model wine*

Application

Since the presence and relevance of thiols has been already established for a number of years in Sauvignon Blanc wines and recently demonstrated in South African Chenin Blanc wines, the proposed method was applied to determine thiol levels in young commercial South African wines from the two cultivars. 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. All samples were analysed within two months after bottling (vintage 2016).

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

Conclusions

✓ The method is suitable for the analysis of thiols in white wine
✓ Using MS/MS detection improved LOD/LOQ levels compared to the previously published ETP method
✓ Levels of thiols in young South African SB and CB are in the same range: 3MH 178-904 ng/L and 99-1124 ng/L and 3MHA 23-151 ng/L and 5-253 ng/L in Sauvignon Blanc and Chenin Blanc wines, respectively
✓ 4MMP was present in Sauvignon Blanc up to 21.9 ng/L, but in none of the Chenin Blanc samples

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