Distribution of enantiomers of 3-sulfanylhexan-1-ol and its acetate in wine determined by HPLC-MS/MS

Liang Chen[1], Dimitra L. Capone[2,3], David W. Jeffery[1]

[1] Department of Wine and Food Science, The University of Adelaide (UA), PMB 1, Glen Osmond SA 5064, Australia

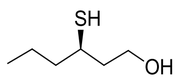
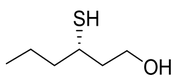
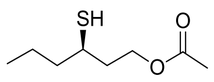
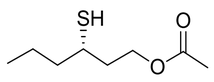
[2] The Australian Wine Research Institute (AWRI), PO Box 197, Glen Osmond, South Australia 5064, Australia

[3] Present address: Australian Research Council Training Centre for Innovative Wine Production, The University of Adelaide

Email: david.jeffery@adelaide.edu.au

Introduction

- Passionfruit and citrus aromas that typify Sauvignon blanc wines are primarily due to polyfunctional thiols, especially 3-sulfanylhexan-1-ol (3-SH) and related 3-sulfanylhexyl acetate (3-SHA).
- These potent volatiles, found at ng/L concentrations, are present as pairs of enantiomers that differ in odour quality and detection threshold (Table 1).[1]

Table 1	(*R*)-3-SH	(*S*)-3-SH	(*R*)-3-SHA	(*S*)-3-SHA
Structure				
Threshold[a]	50	60	9	2.5
Sensory description	grapefruits, citrus peel	passion fruit	passion fruit	boxwood
Concentration[b]	275–1031 (2998)[c]	368–1129 (4396)[c]	not reported	not reported

[a] ng/L, in model wine media. [b] ng/L, commercial Sauvignon blanc (n=12) and Chardonnay (n=1) wines.

[c] Values in parentheses are for a botrytised Sauvignon blanc wine.

- Different enantiomer ratios can affect the sensory properties of wines,[2] but except for a 2006 study,[3] little is known about factors that impact the distribution profiles of the enantiomers.
- We recently developed a stable isotope dilution analysis (SIDA) method for thiol analysis involving in situ derivatisation with 4,4'-dithiodipyridine (DTDP) followed by SPE and HPLC-MS/MS analysis,[4] and have now adapted that to chiral HPLC.[1]
- This necessitated synthesis of authentic derivatives and chiral column screening with further method validation on the chosen phase, as outlined in Figure 1.

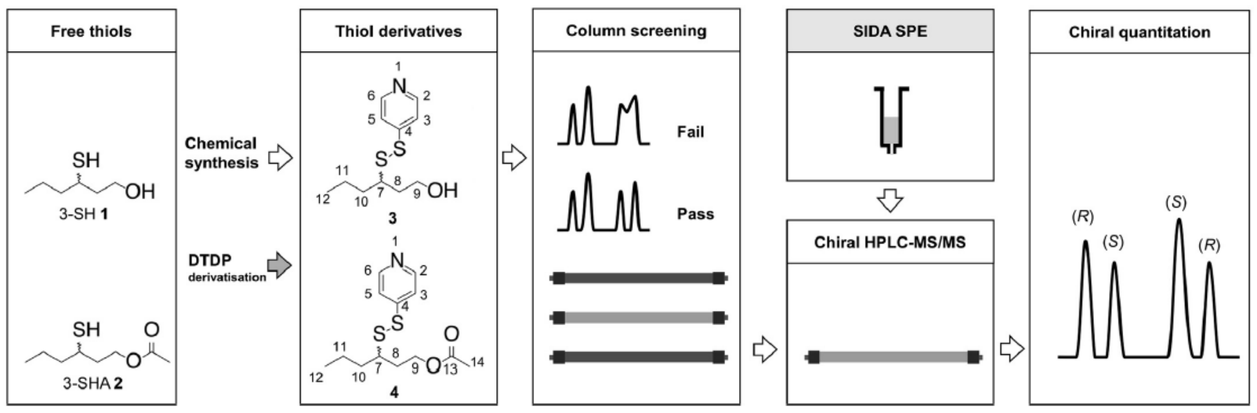
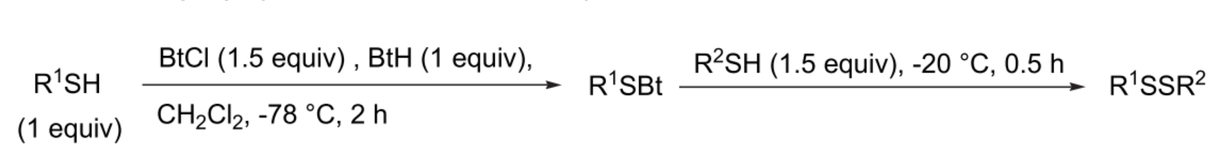


Figure 1. Approach to resolving and determining enantiomers of 3-SH and 3-SHA in wine using chemical synthesis of thiol–thiopyridine derivatives, chiral column screening, derivatisation in wine and SPE clean-up, and precise quantitation by SIDA with chiral HPLC-MS/MS.[1]

Methods[1]

- Authentic derivatives were prepared from 3-SH and 3-SHA for chiral column screening using a one-pot procedure for asymmetric disulfide formation mediated by 1-chlorobenzotriazole (BtCl). Products were purified by SiO_2 chromatography and characterised by HPLC-MS/MS, HRMS and NMR.



- Validation of an optimised method was conducted with Lux Amylose-1 using freshly prepared 5 mM aqueous ammonium bicarbonate and MeCN. MS data were recorded in multiple reaction monitoring (MRM) mode with MRM transition pairs for analytes and deuterated internal standards as previously reported.[4]
- Elution order was determined using pure enantiomers spiked in model wine and derivatised with DTDP. Peak identity was also confirmed by fortifying pure (*R*)-enantiomers in a charcoal-stripped Sauvignon blanc wine spiked with racemic mixtures of 3-SH (1000 ng/L) and 3-SHA (200 ng/L).

Results and Discussion

- Expense of chiral HPLC columns discounted a trial and error approach to selecting a column. Authentic standards of thiol derivatives were prepared and used for chiral column screening by PhenoLogix to determine the most appropriate chiral stationary phases in an LC-MS compatible format (but with 4.6 mm i.d.).
- Amylose-1 (comprising amylose 3,5-dimethylphenylcarbamates, ADMPC) with isocratic elution achieved full enantioseparation of 3-SH and 3-SHA but required a 50 min run time.
- With some modification of the HPLC conditions, all analytes could be eluted in under 24 min, after which the elution order of enantiomers was determined (Figure 2).
- The enantiomeric bias of ADMPC for 3-SH (selectivity factor α = 5.69) was noteworthy and extreme cases of HPLC enantioseparation on ADMPC have previously been reported for other compounds.
- Recoveries in white, red, rosé and model wine ranged from 90–111%, precision was < 8%, and LOD values were < 0.7 ng/L.

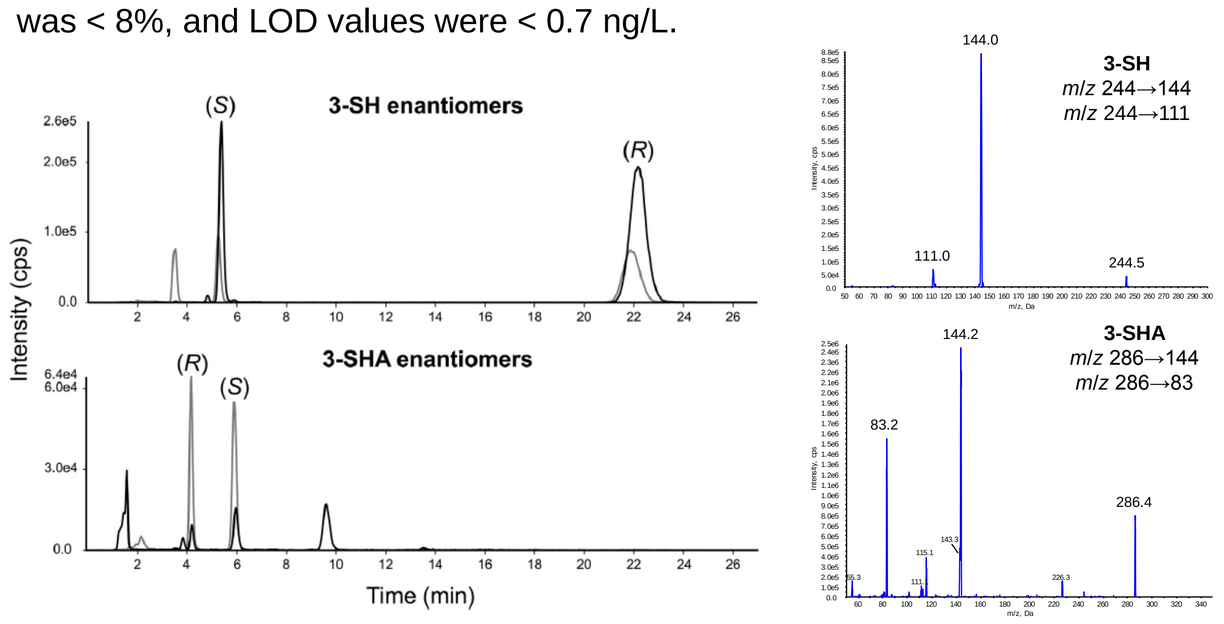


Figure 2. MRM chromatograms (left, black line = analyte, grey line = labelled IS) and product ion mass spectra (right) of enantiomers of 3-SH and 3-SHA isolated from a Sauvignon blanc wine (as their derivatives) using the optimised chiral HPLC-MS/MS method.[1]

- The method was applied to a number of commercial wines (Figure 3) and enantiomer ratios were determined to be an average of 52:48 for (*S*)-:(*R*)-3-SH and 60:40 for (*S*)-:(*R*)-3-SHA. Botrytised wine showed a higher proportion of the (*S*)-enantiomers.

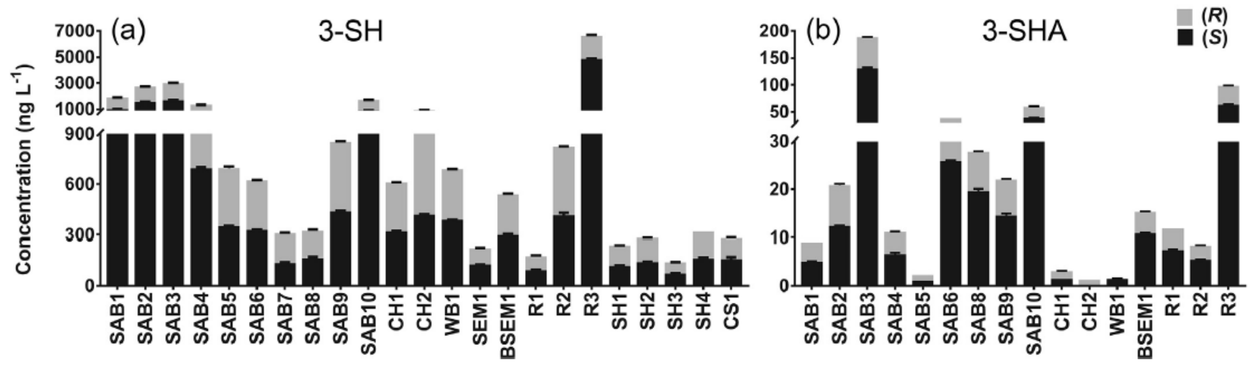


Figure 3. Concentrations of enantiomers of (a) 3-SH and (b) 3-SHA in a selection of commercial wine samples. SAB, Sauvignon blanc; CH, Chardonnay; WB, white blend; SEM, Semillon; BSEM, botrytised Semillon; R, rosé; CS, Cabernet Sauvignon.[1]

Conclusions

- This study presents a new chiral HPLC-MS/MS SIDA method for quantitation of the enantiomers of 3-SH and 3-SHA in wine.
- The method was applied to a range of commercial wines, showing that enantiomers of 3-SH were rather evenly distributed but those of 3-SHA usually favoured (S)-3-SHA. Botrytised wines tended to show a ratio of 70:30 of (*S*)-:(*R*)-enantiomers.
- Additional studies have since been undertaken to further explore enantiomer profiles in relation to grape-derived thiol precursor diastereomers.[5]

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