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

Liang Chen¹, Dimitra L. Capone², ³, David W. Jeffery¹

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>
<thead>
<tr>
<th>Structure</th>
<th>(R)-3-SH</th>
<th>(S)-3-SH</th>
<th>(R)-3 SHA</th>
<th>(S)-3 SHA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Threshold⁴</td>
<td>50</td>
<td>60</td>
<td>9</td>
<td>2.5</td>
</tr>
<tr>
<td>Sensory description⁵</td>
<td>passion fruit</td>
<td>passion fruit</td>
<td>boxwood</td>
<td></td>
</tr>
</tbody>
</table>

⁴ ng/L, in model wine media. ⁵ ng/L, commercial Sauvignon blanc (n=12) and Chardonnay (n=1) wines.

Values in parentheses are for a botrytised Sauvignon blanc wine.

• Different enantiomer ratios can affect the sensory properties of wines,⁶ but except for a 2006 study⁷ 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’-dithiodipyrindine (DTDP) followed by SPE and HPLC-MS/MS analysis,⁸ 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 thioli-thiopyridine derivatives, chiral column screening, derivatisation in wine and SPE clean-up, and precise quantitation by SIDA with chiral HPLC-MS/MS/MS.¹](image)

Methods¹¹

• 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 (BClO). Products were purified by SiO₂ chromatography and characterised by HPLC-MS/MS, HRMS and NMR.

R²S⁻¹CH₂Cl₂ -78 °C, 2 h

BClO (1.5 equiv), BH (1 equiv), 2 h

R²S⁻¹

CH₂Cl₂ -78 °C, 2 h

R²S⁻¹ (1 equiv)

• 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.⁹

• 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 PhenolLogix 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 enantioreparation 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 enantioreparation 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.¹¹](image)

• 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 (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.¹¹](image)

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.¹¹