Determination of sotolon content in South African white wines by two novel HPLC-UV and UPLC-MS methods

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Abstract

Sotolon has been reported to play an important role in the atypical ageing and aroma character of many wines. A number of analytical techniques for sotolon analysis in wine

have been reported, but these often require extensive sample preparation. In this work we report a HPLC-UV method and a novel UPLC-MS method to determine sotolon concentrations in white wines with little sample preparation applied for the first time for the evaluation of sotolon levels in South African wines. The validation showed that the instrumental methods had good accuracy, repeatability and linearity, but the UPLC-MS method proved more sensitive. For both methods, quantification limits were lower than the sotolon odour threshold in wine (10 µg/L), 0.86 µg/L and 0.013 µg/L, for HPLC-UV and UPLC-MS methods, respectively. Sotolon levels in 65 South African white wines were often found to be lower than the reported odour threshold, with the highest concentration being 9.11 µg/L. However, for low levels (< 1 µg/L), unknown interferences in certain wines led to sotolon not being quantified with the HPLC-UV method, which made the UPLC-MS method more suitable.

Keywords: sotolon, white wine, liquid chromatography

1. Introduction

Sotolon (3-hydroxy-4,5-dimethyl-2(5)-furanone) is a powerful flavour compound with an intense spicy /curry odour (Girardon, Sauvaire, Baccou & Bessiere, 1986). Sotolon has an aroma associated with aged sake (Takahashi, Tadenuma & Sato, 1976), roasted coffee (Blank, Sen & Grosh, 1992), fenugreek (Girardon *et al*., 1986) and sugar cane (Tokitomo, Kobayashi, Yamanishi & Murahi, 1980). Sotolon has been identified and quantified in different wines, such as botrytised (or noble rot) wines (5–20 µg/L) (Masuda, Okawa,

47 Nishimura & Yunome, 1984), port (5–958 µg/L) (Silva Ferreira, Barbe & Bertrand, 2003),
48 vin Javen (120–268 µg/L) (Pham, Guichard, Schlich & Charpentier, 1995), sherry (0–500
49 µg/L) (Martin, Etiévant, Le Quéré & Schlich, 1992) and Madeira (0–2 000 µg/L) (Camara,
50 Marques, Alves & Silva Ferreira, 2004), and in barrel-aged white wines (0–140 µg/L)
51 (Lavigne, Pons, Darriet & Dubourdieu, 2008). Its odour threshold is extremely low: 0.02
52 µg/L in air (Blank, Lin, Fumeaux, Welti & Fay, 1996), 0.3 µg/L in water (nasal detection)
53 (Blank et al., 1996) and 10 µg/L in white wine (human perception) (Guichard, Pham &
54 Etiévant, 1993). Although it is associated to a typical flavour note in Madeira, port, sherry
55 and long-aged sweet wines, sotolon is considered to be one of the compounds responsible
56 for the atypical ageing and oxidative off-flavour in dry white wines when its concentration is
57 higher than the odour threshold (Du Toit, Marais, Pretorius & Du Toit, 2006).

58 Several pathways for the formation of sotolon are reported in the literature. It can be
59 produced by thermal degradation of intermediate compounds of the Maillard reaction
60 (Blank et al., 1996; Guerra & Yaylayan, 2011; Hofmann & Schieberle, 1997). Cutzach,
61 Chatonnet and Dubourdieu (1999) showed a pathway for the formation of sotolon via aldol
62 condensation between α-keto butyric acid and acetaldehyde. Konig, Gutsche, Hartl,
63 Hubscher, Schreier and Schwab (1999) explained that sotolon is produced by the reaction
64 between ethanol and ascorbic acid. During winemaking and ageing, sotolon formation is
65 affected by chemical and physical factors such as the presence of oxygen (Cutzach et al.,
66 1999; Lavigne et al., 2008), the reducing sugar concentration (Camara et al., 2004),
67 storage temperature and time (Cutzach et al., 1999), and concentrations of certain
68 antioxidants (e.g. sulphur dioxide, glutathione) (Dubourdieu & Lavigne, 2004).

69 Due to the number of physical and chemical factors affecting the formation of sotolon in
70 wine, this compound was suggested as a chemical marker of the shelf-life for dry white

wine (Lavigne & Dubourdieu, 2004). However, the levels of sotolon in South African white wine have not been investigated before.

Several analytical techniques have been reported for the determination of sotolon in wine, including Multi-Dimensional Gas Chromatography (MDGC-MS) and High-Resolution GC (HRGC-MS) (Konig et al., 1999); High-Resolution GC Olfactometry (HRGC-MS-O) (Escudero, Cacho & Ferreira, 2000); GC Olfactometry (GC-O) (Silva Ferreira et al., 2003); Two Dimensional Capillary GC (2D-CGC) (Martin & Etiévant, 1991; Dugo et al. 2014); GC-MS (Pons, Lavigne, Landais, Darriet & Dubourdieu, 2010; Castro et al. 2014; Zea et al. 2013); Two Dimensional GC (2D-GC) (Martin et al., 1992); and High Pressure Liquid Chromatography (HPLC-UV) (Guichard et al., 1993). Moreover, the sotolon concentration in wine is usually low and the compound has high boiling temperature (184°C), both affecting negatively the sensitivity of the analytical methods based on head space sampling technique (DHS and SPME) ((Ferreira et al. 2000); Ferreira, Jarauta, Lopez & Cacho, 2003). The sample preparation requires both an extraction step (liquid/liquid extraction or solid phase extraction (SPE)) followed by a concentration step prior the chromatographic separation (Cutzach et al., 1999; Konig et al., 1999). Generally, these reported methods use either instrumentation that is not standard in oenology laboratories or long extraction time (Escudero et al., 2000), and substantial volumes of both sample and solvents (Takahashi, Tadenuma & Sato, 1976; Konig et al., 1999; Schneider, Baumes, Bayonove & Razungles, 1998).

The two main aims of this study thus were to develop, validate and compare two fast and reproducible chromatographic methods (UPLC-MS and HPLC-UV) for sotolon analysis in wine, and to use these methods to assess sotolon levels in South African white wines in order to understand the occurrence of atypical aging causing a decrease of wine shelf-life.

2. Materials and Methods

2.1 Chemicals

4,5-Dimethyl-3-hydroxy-2,5-dihydrofuran-2-one (≥ 97%), dichloromethane (≥ 99.8%), sodium chloride (≥ 99.5%), methanol (≥ 99.9%), acetonitrile LC-MS CHROMASOLV® (≥ 99.0%), iso-propanol LC-MS CHROMASOLV® (≥ 99.0%) and anhydrous sodium sulphate (≥ 99.0%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). UPLC water was obtained from a Milli-Q filtration system (Millipore Filter Cor., Bedford, MA, USA). Polyvinylpolypyrrolidone (PVPP) resin was purchased from Dal Cin Gildo spa (Sesto San Giovanni, Milano, Italy). The model wine contained 12% (v/v) ethanol and 5 g/L of tartaric acid, and the pH was adjusted to 3.5 with sodium hydroxide (Sigma-Aldrich St. Louis, MO, USA).

2.2 White wine samples

Sotolon analysis was carried out on 70 commercial South African white wines. The commercial wines were produced from ten different grape cultivars (Sauvignon blanc, Chardonnay, Chenin blanc, Viognier, Semillon, Grenache blanc, Pinot Grigio, Colombard, Gewurztraminer and Rhine Riesling) and sixteen different vintages (from 1983 to 2013). The wine samples coded by number (1 to 65) were sourced directly from local cellars, while the wines coded by letter (a to e) were stored for two years at 37°C.

2.3 Sample preparation

The sample preparation was done according to Gabrielli (2014a, 2014b). The equivalent of 3 g/L NaCl was added to 30 mL of white wine. The wine was extracted twice with 20 mL

dichloromethane for 10 min with stirring. The organic phases were combined and 2 g anhydrous Na_2SO_4 was added to remove traces of water. Dichloromethane was evaporated to dryness under a gentle nitrogen stream, and the dry material was re-dissolved in 2 mL of 5% methanol solution. The concentrated extract was further purified with 50 mg of PVPP resin by dispersion in the sample. The solution was filtered (0.22 µm PVDF, Millipore, MO, USA) before injection.

2.4 UPLC–MS/MS and HPLC-UV analysis

UPLC-MS separations were performed with a Waters Acquity H Class UPLC system connected to a Waters Xevo triple quadrupole mass spectrometer (Waters, Milford, MA, USA). The column used was a BEH C18, 2.1 x 100 mm, 1.7 µm (Waters, Milford, MA, USA). Data were acquired in multiple reaction monitoring (MRM) mode, electrospray positive ionisation, precursor ion at *m/z* 129, and the product ions at *m/z* 55 and 83, using a collision energy of 20 V and 15 V, respectively. A cone voltage of 20 V was used. The desolvation temperature was set at 400°C, and the desolvation gas was 900 L/h. A capillary voltage of 3.5 kV was used and the rest of the MS settings were optimised for best sensitivity. The mobile phases were (A) 1% formic acid in water and (B) methanol:acetonitrile:iso-propanol (49:49:2), and the flow rate was 0.4 mL/min. The injection volume was 3 µL and the column temperature was at 30°C.

HPLC-UV separations were performed with an Agilent 1260 Series system fitted with a diode array detector (Agilent, Palo Alto, CA, USA). The column used was a Kinetex C18 100 x 3 mm x 2.6 µm, from Phenomenex (Torrence, CA, USA). The sotolon was detected and quantified at 235 nm. The mobile phases used were (A) water and (B) methanol, and the flow rate was 0.45 mL/min. The injection volume was 20 µL and the column temperature was 30°C. The gradients are reported in **Table 1**.

2.5 Validation procedure

The validation of the methods was carried out with respect to qualitative (selectivity) and quantitative (linearity, LOD, LOQ, repeatability and accuracy) parameters.

2.5.1 Selectivity

Selectivity was tested by spiking model wine and a young dry white wine with 10 µg/L sotolon, performing the sample preparation procedure and the separation, and comparing the chromatograms. In this way, the selectivity of the method was evaluated by comparing the results obtained for the detection of sotolon in the absence and presence of possible interferences originating from the white wine matrix.

2.5.2 Linearity

The linearity interval tested was 5 to 50 µg/L at six concentration levels, in young dry white wine and in model wine, with extractions done in duplicate. The linearity correlation coefficients (R^2) were calculated from the regression analysis. The limit of detection (LOD) and limit of quantitation (LOQ) were calculated as the lowest concentration of analyte in a sample that resulted in a signal-to-noise ratio of 3 (LOD) and 10 (LOQ), respectively. The baseline noise was calculated by the software (MassLynx, Waters).

2.5.3 Accuracy (recovery test)

166 Accuracy was measured for two levels of sotolon, specifically 10.7 µg/L and 21.5 µg/L.
167 Spiked model wine (at both concentrations), white wine (blank) and the same white wine,
168 spiked (at both concentrations) were extracted in duplicate.

169

170 *2.5.4 Precision*

171 Precision was expressed as repeatability (intra-day measurements) and intermediate
172 precision (inter-day measurements). The model wine and white wine were spiked with two
173 levels of sotolon corresponding to a medium-low (10.7 µg/L) and medium-high (21.5 µg/L)
174 concentrations. The extractions were done in triplicate, and the intermediate precision
175 calculated over three days. For the instrumental repeatability, spiked white and model
176 wines containing 21.5 µg/L of sotolon were injected five times. The relative standard
177 deviation (RSD) values were calculated for the peak areas and retention times.

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179

180 **3. Results and Discussion**

181 3.1 Sample preparation

182 Compared to the analytical procedures proposed by other authors, the method described
183 above presents sample preparation steps (extraction and purification) that are rapid,
184 improve sensitivity and are easy to apply in practice. Other authors have proposed
185 procedures that use long extraction times (up to 48 h) (Escudero et al., 2000) and larger
186 volumes of wine (up to 100 mL) (Lavigne et al., 2008; Pons et al., 2010) and solvents (up
187 to 250 mL) (Konig et al., 1999; Schneider, Baumes, Bayonove & Razungles, 1998).

188

189 3.2 Comparison of the validation results for the UPLC-MS and HPLC-UV methods

190 The comparison between the two instrumental methods was done using the validation
191 parameters for each method, namely selectivity, linearity, precision and accuracy.

192 Selectivity (lack of interferences) was evaluated by comparing the sotolon peak in the
193 presence and in the absence of interferences from the matrix. The UPLC-MS and HPLC-
194 UV chromatograms from samples of spiked model wine and dry white wine are reported in
195 **Figure 1** and **2.** The retention times for sotolon were 2.2 min and 5.7 min using the UPLC-
196 MS and HPLC-UV separation respectively. Although much less interference was observed
197 with the UPLC-MS method and the baseline noise is lower, sotolon could also be
198 measured by HPLC-UV in the absence of (potential) interference from the matrix.

199 Both methods showed good linear response, as measured by the correlation coefficients,
200 R^2 (Table 2). The concentration range chosen is in accordance with the sotolon
201 concentration values previously reported in wine (Camara et al., 2004; Lavigne et al.,
202 2008; Pons et al., 2010).

203 The UPLC-MS method had an LOD of 0.001 µg/L and the LOQ was 0.003 µg/L in wine.
204 The HPLC-UV method had LOD and LOQ values for wine of 0.259 µg/L and 0.862 µg/L,
205 respectively. The odour threshold of sotolon in wine is approximately 10 µg/L (Guichard et
206 al.,1993). To have practical application in sensorial wine investigations, an analytical
207 method for sotolon should be able to measure concentrations below the odour threshold.
208 Both methods detected sotolon at levels less than the odour threshold in white wine thus
209 allowing for its determination prior to the defect being perceived by sensory analysis.
210 Moreover, the LOQs were less than LOQ values previously reported in the literature.
211 Guichard et al. (1993) reported an LOD of 10 µg/L in Vin Jaune, Vin de Paille and Tokai by
212 a HPLC-UV method, whereas Camara et al. (2004) reported an LOD of 1.2 µg/L in
213 Madeira wines using GC/MS. Lavigne at al. (2008) reported an LOD of 1.2 µg/L in dry

white wines with a GC/MS method, while Ferreira, Jarauta, López and Cacho (2003) reported an LOD of 0.84 µg/L in white wines by GC/MS. As can be seen from Table 2, the values for LOD and LOQ in the model wine were higher than in white wine samples; this could be attributed to a higher extraction yield from the wine than from the model wine. The reason for this is as yet unclear, but could be investigated in a follow-up study.

Precision is a measure of the agreement between test results from multiple, repeated procedures on a series of standards. Therefore it is important to evaluate both the precision of the sample preparation procedure and that of the instrumental method. The concentrations chosen for testing the precision were 10 and 20 µg/L – the first is close to the odour threshold and the second is two times higher. The RSD% was calculated for peak areas and the values obtained are shown in Table 3. There was no observable trend for the inter-day determination. Generally, the repeatability for the wine sample was better than for the model wine. Repeatability and intermediate precision are acceptable for the determination of sotolon, but could be improved if an internal standard was included in the procedure.

Precision was measured for the instrumental method also, and %RSD calculated for peak areas and retention times. For the HPLC-UV method, the %RSD for peak areas was 1.46 and 0.32 for the wine and model wine, respectively. For the UPLC-MS method, the %RSD for peak areas was 5.45 and 1.94 for the wine and model wine, respectively. For retention times, the %RSD was 0.23 for the HPLC-UV method and 0.23 for the UPLC-MS method.

Accuracy measures the amount of analyte that is quantified relative to the amount present in the sample. In other words, recovery tests will indicate the amount of analyte quantified in the presence and in the absence of matrix interferences. The recovery values (Table 3) were acceptable for both methods (over 75%), but the use of an internal standard would have improved accuracy.

In comparison with previously reported GC-MS and HPLC methods (Camara et al., 2004; Guichard et al., 1993; Lavigne et al., 2008), the UPLC-MS method showed improved sensitivity and speed for sotolon quantification in white wines. The HPLC-UV method was shown to be acceptable for the determination of sotolon, even when present below the odour threshold. However, the much lower LOD and LOQ of the UPLC-MS method makes it more appropriate not only for the measurement of sotolon at concentrations at or around the odour threshold (an important marker from a sensory point of view) but also for metabolic studies, where much lower concentrations could be of interest. The majority of the analytical methods described in the literature for sotolon determination in wine are based on GC-MS (Camara et al., 2004; Lavigne et al., 2004; Oliveira e Silva et al., 2008; Silva Ferreira et al., 2003;). The setting up of an analytical method allowing sotolon determination using HPLC-UV could represent an alternative tool for oenological and cellar laboratories that do not have routine access to GCMS.

3.3 Sotolon quantification in white wine

The methods developed were successfully applied for the determination of sotolon levels in 65 South African white wines (sweet and dry). The sotolon concentrations as determined with the UPLC-MS and HPLC-UV methods are shown in Table 4.

Using the UPLC-MS method, the highest sotolon concentrations were found in wine 49 (9.11 µg/L, dry, 2010) and 56 (8.72 µg/L, sweet, 1999). In both these wines, sotolon was close to the reported odour threshold. Using this method, sotolon was not detected in 15 other wines, while it was lower than the odour threshold for most of the wines analysed.

The HPLC-UV method also detected the highest sotolon concentration in wine 49 (8.13 µg/L), even though it was lower (-10.8%) than the concentration measured by ULPC-MS. Sotolon was not detected in 42 wines using this method, 27 more than when using the

264 UPLC-MS method, and was not quantifiable in seven wines, including wine 56. As
265 expected, sotolon could be measured in more wines by UPLC-MS, due to the lower LOQ
266 of the method, than by HPLC-UV. Moreover, due to the better selectivity of MS than UV,
267 the measurement of sotolon with the UPLC-MS method did not suffer from interference.
268 The levels of sotolon marked as “not quantifiable” (Table 4) are for samples in which the
269 measurement was impeded by the presence of an interfering peak, which needs further
270 investigation.

271 The sotolon content in wine is reported to be related to the winemaking conditions, e.g.
272 oxidative/reducing conditions, barrel ageing (Cutzach et al., 1999; Schneider et al., 1998),
273 as well as the sugar content in the wine (Camara et al., 2004). However, the sotolon level
274 in most of the South African wines was less than the odour threshold, even in wines
275 containing sugar and/or those older than 10 years. Thus, these levels were lower than
276 those previously reported (Camara et al., 2004; Guichard et al., 1993; Martin et al., 1992;
277 Oliveira e Silva et al., 2008; Silva Ferreira et al., 2003). Dagan, Schneider, Lepoutre and
278 Baumes (2006) also found sotolon levels in different older wines to be less than the odour
279 threshold. However, five additional wines stored at a higher temperature (37°C) for two
280 years (wines a to e) had sotolon levels 1.5 to 3 times higher than the odour threshold
281 (Table 4). This finding confirms the significant effect that temperature has on sotolon in
282 white wine (Cutzach et al., 1999).

283 This study, although performed on a limited number of white wines, indicates that sotolon
284 does not occur at concentrations higher than the odour threshold in most South African
285 wines and therefore has a limited role in atypical ageing character. However, sotolon may
286 contribute to the atypical ageing character of South African white wines by acting in a
287 synergistic manner with aroma-related compounds such as maltol, furaneol, homofuraneol
288 and cyclotene (Dagan et al., 2006). This is a matter that needs further attention.

Conclusions

The analytical methods developed used sample preparation steps that were quicker and easier to apply in practice than other previously reported methods. The validation showed that the instrumental methods (UPLC-MS and HPLC-UV) had good accuracy, repeatability and were linear. The UPLC-MS method showed better sensitivity, but the repeatability was best for the HPLC-UV method. Even so, both methods were proven to be suitable for the determination of sotolon below the sensory odour threshold in most white wines. The two methods were used successfully for the screening of commercial South African wines. In general, sotolon does not occur in South African white wines at levels greater than the odour threshold. However, sotolon levels can be increased in white wines stored for prolonged periods at high temperatures.

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402 Figure 1. Sotolon peak from spiked model wine (bottom trace) and spiked white wine (top
403 trace) in UPLC-MS.

404

405 Figure 2. Sotolon peak from spiked model wine (bottom trace) and spiked white wine (top
406 trace) in HPLC-UV. Detection at 235 nm.

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