

1 **Analysis of volatile thiols in alcoholic beverages by simultaneous derivatization/extraction**
2 **and liquid chromatography-high resolution mass spectrometry**

3

4 Stefania Vichi^{a,*}, Nuria Cortés-Francisco^a, Josep Caixach^b

5 ^a *Food Science and Nutrition Department, XaRTA (Catalonian Reference Network on Food*
6 *Technology), University of Barcelona, Food and Nutrition Torribera Campus, Av. Prat de la Riba,*
7 *171. 08921, S.ta Coloma de Gramenet, Spain.*

8 ^b *Mass Spectrometry Laboratory / Organic Pollutants, IDAEA-CSIC. Jordi Girona, 18-26. 08034*
9 *Barcelona, Spain.*

10

11 **Running title:** Volatile thiols in alcoholic beverages by derivatization and LC-HRMS

12

13

14

15

16 *Corresponding author

17 Phone: (+34) 93 403 3794; Fax: (+34) 93 403 5931; e-mail: stefaniavichi@ub.edu

18

19

20 **ABSTRACT**

21 A simultaneous derivatization/extraction method followed by liquid chromatography-
22 electrospray-high resolution mass spectrometry for the determination of volatile thiols in
23 hydroalcoholic matrixes was optimized and used to identify and quantify volatile thiols in wine
24 and beer samples. The method was evaluated in terms of sensitivity, precision, accuracy and
25 selectivity. The experimental LOQs of eleven thiols tested ranged between 0.01 ng/L and 10
26 ng/L. Intra-day relative standard deviation (RSD) was in general lower than 10% and inter-day
27 RSD ranged between 10% and 30%. Recovery in the model and real matrixes ranged from 45%
28 to 129%. The method was then applied for the analysis of four white wines and six beers. Five
29 out of the eleven reference thiols were identified and quantified in the samples analysed. The
30 non-target approach, carried out by monitoring the diagnostic ion at m/z 275.9922
31 $[C_{13}H_{10}ONSe]^+$ in the fragmentation spectrum, allowed detecting, in the same samples,
32 fourteen non-target thiols.

33

34 **Keywords:** wine, beer, thiols, derivatization, high resolution mass spectrometry (HRMS),
35 HRMS/MS, Orbitrap.

36

37 1. INTRODUCTION

38 Volatile sulfur compounds are major contributors to several food and beverage aromas
39 (McGorin, 2011; Vermuelen Gijs & Collin, 2005). Due to their extremely low odor thresholds,
40 they have a significant sensory impact even at very low concentrations. In particular, volatile
41 thiols are well known to be powerful odorants in beverages such as wine (Tominaga,
42 Baltenweck-Guyot, Peyrot des Gachons & Dubourdieu, 2000; Kotseridis & Baumes, 2000) and
43 beer (Vermeulen, Lejeune, Tran & Collin, 2006; Hugues, 2009). Among wine varietal thiols, 4-
44 mercapto-4-methyl-pentan-2-one, 3-mercaptohexan-1-ol (3MH) and 3-mercaptohexyl acetate
45 (3MHA), are responsible for box tree, exotic fruit and grapefruit aromas, respectively, in wines
46 made from Sauvignon Blanc, Colombard, Scheurebe, Merlot and Cabernet Sauvignon cultivars,
47 among others (Tominaga et al., 1999; Schneider, Charrier, Razungles & Baumes; 2006;
48 Kotseridis & Baumes, 2000; Guth, 1997; Darriet, Tominaga, Lavigne, Boidron & Dubourdieu;
49 1995; Bouchilloux, Darriet, enry, Lavigne-Cruege & Dubourdieu, 1998). Other thiols such as 2-
50 furanmethanethiol (2FMT), 2-methyl-3-furanthiol (2M3FT), and benzenemethanethiol, have
51 been associated with the empyreumatic notes of aged wines (Blanchard, Tominaga &
52 Dubourdieu, 2001; Tominaga, Blanchard, Darriet & Dubourdieu, 2000; Tominaga, Guimbertau
53 & Dubourdieu, 2003). Furthermore, several polyfunctional thiols have been reported in beer,
54 usually associated with sensory defects. In particular, 3-methyl-2-buten-1-thiol (3MBT) imparts
55 the well-known lightstruck and “skunky” off flavors (Huvaere, Andersen, Skibsted, Heyerick &
56 Keukeleire, 2005; Goldstein, Rader & Murakami, 1993; Lermusieau & Collin, 2003), while 4-
57 mercapto-4-methyl-2-pentanone (Cosser, Murray & Holzapfel, 1980) and 3-mercapto-3-
58 methylbutyl-formate (Schieberle, 1991) induce a “ribes, catty” odor. Recently, 3-mercapto-2-
59 methylbutanol and 2-mercapto-3-methylbutanol were associated with onion-like notes in beer
60 (Vermeulen et al., 2006). Moreover, 2M3FT (Lermusieau, M. Bulens & Collin, 2001) and 3MH

61 are often present in fresh lager beers, although their sensory impact seems of little relevance
62 ([Vermeulen et al., 2006](#)).

63 Finally, highly volatile thiols, such as hydrogen sulfide (H₂S), methanethiol, ethanethiol and
64 propanethiol, have been identified as responsible for putrefaction, garlic, onion or rotten egg-
65 like notes, in beer ([Hugues, 2009](#)) and in wine, as reviewed by [Mestres et al. \(2000\)](#).

66 The analytical assay of volatile thiols in food and beverages is particularly difficult due to the
67 complexity of the matrixes, together with the typically low concentrations and high reactivity
68 of the thiols. The most widely used analytical methods are based on the liquid–liquid
69 extraction of thiols from a wine or beer matrix, followed by derivatization with *p*-
70 hydroxymercurybenzoate ([Vermeulen et al., 2006](#); [Tominaga, Murat & Dubourdieu, 1998](#)) or
71 by reversible covalent chromatography ([Schneider, Kotseridis, Ray, Augier & Baumes, 2003](#)).

72 More recently, 2,3,4,5,6-pentafluorobenzyl bromide (PFBBBr) has been used to derivatize thiols
73 on solid-phase microextraction (SPME) fibers ([Mateo-Vivaracho, Cacho & Ferreira, 2007](#)) or in
74 the wine matrix, followed by isolation of the derivatives either via solid-phase extraction (SPE)
75 ([Mateo-Vivaracho, Cacho & Ferreira, 2008](#)) or SPE followed by SPME ([Rodríguez-Bencomo,](#)
76 [Schneider, Lepoutre & Rigou, 2009](#)). The best quantification performance achieved with these
77 methods uses stable isotopic dilution, which involves the synthesis of deuterated thiols.

78 These procedures require relatively large sample amounts, they are laborious and time-
79 consuming, and some of them involve several sample-manipulation steps during which volatile
80 thiols can be lost or undergo degradation.

81 In order to devise a simple, reliable, selective and sensitive method for the analysis of volatile
82 thiols at trace levels in hydroalcoholic matrices such as wine and beer, a simultaneous
83 derivatization/extraction method followed by liquid chromatography-electrospray-high
84 resolution mass spectrometry (LC-ESI-HRMS) was developed using ebselen as the
85 derivatization reagent. This choice was based on recent reports of its high selectivity, fast

86 reaction and high derivatization yield for some biological thiols (Xu, Zhang, Tang, Laskin, Roach
87 & Chen, 2010), and volatile thiols in lipid matrixes (Vichi, Cortés-Francisco & Caixach, 2013).
88 HRMS was chosen to provide the highest chemical selectivity . The analytical conditions were
89 optimized in model systems and real wine and beer samples, both spiked with eleven
90 representative volatile thiols. The method was first evaluated in terms of sensitivity, precision,
91 accuracy and selectivity, and then applied to real samples. Moreover, a non-target approach
92 based on the formation of a diagnostic product ion was evaluated using real wine and beer
93 samples.

94 **2. MATERIAL AND METHODS**

95 **2.1. Chemicals and reagents**

96 Mass spectrometry grade dichloromethane and methanol (MS SupraSolv®) were purchased
97 from Merck (Darmstadt, Germany). Water was of ultrapure milli-Q grade. Ammonium formate
98 was from Sigma-Aldrich (St. Louis, MO, USA). Nitrogen (Alphagaz N₂, purity 99.999%, Air
99 Liquid) was used in the Orbitrap-Exactive as nebulization and fragmentation gas.

100 Ebselen (2-phenyl-1,2-benzisoselenazol-3(2H)-one, purity 98%), absolute ethanol (purity
101 99.8%), ethylenediaminetetraacetic acid (EDTA) (purity 98,5%); 3-mercaptohexyl acetate
102 (3MHA) (purity 98%); 3-mercaptohexanol (3MH) (purity 98%); 3-mercapto-3-methylbutan-1-ol
103 (3MMB) (purity 98%); 2-furanmethanethiol (2FMT) (purity 97%); *p*-mentha-8-thiol-3-one (MT)
104 (purity 98%); 1-hexanethiol (HT) (purity 98%); 4-methoxy- α -toluenethiol (IS, purity 90%) were
105 purchased by Sigma-Aldrich (St Louis, MO, USA). 4-mercapto-4-methylpentan-2-ol (4MMP)
106 (purity 98%), 4-methoxy-2-methyl-2-butanethiol (4MMB) (purity 98%); 3-mercaptohexyl
107 hexanoate (3MHH) (purity 98%); 1-phenylethyl mercaptan (1PEM) (purity 98%) were from
108 Endeavour Speciality Chemicals Ltd (Northants, UK). 3-methyl-2-butene-1-thiol preparation kit
109 was provided by Toronto Research Chemicals Inc. (North York, Canada). Molecular structures
110 of the reference thiols are shown in **Fig. S1 (Supplementary information)**.

111 **2.2. Model wine and model beer**

112 Model wine was prepared according to [Labanda et al. \(2009\)](#), by dissolving 5 g/L of glycerol, 10
113 g/L of glucose, 5 g/L of tartaric acid, 10 mg/L of albumin, 300 mg/L of pectin, 100 mg/L of
114 tannic acid, 150 mg/L of sodium metabisulfite and 120 mL of absolute ethanol in deionized
115 water. The pH of the model wine was 3.0.

116 Model beer was prepared according to [Eagles & Wakeman \(2002\)](#) with some modifications:
117 1.5g/L of glycerol, 1.5 g/L of maltose 100 mg/L of citric acid, 100 mg/L of albumin, 300 mg/L of
118 pectin, 50 mg/L of tannic acid and 50 mL/L of absolute ethanol in deionized water. The pH of
119 the model beer was 4.2.

120 **2.3. Wine and beer samples**

121 The method was applied to distinct commercial samples of wine and beer provided by local
122 retailers. Wine samples were: Albariño (2012) Denomination of Origin (D.O.) Rias Baixas (Spain)
123 (sample W1); Sauvignon Blanc (2012) D.O. Rueda (Spain) (W2); Riesling (2012) D.O. Penedés
124 (Spain) (W3); Sauvignon blanc/gewürztraminer (2012) D.O. Penedès (Spain) (W4). Two lager
125 beer samples exposed to light (B1, B2), one lager (B3), one double-malt (B4), one alcohol free
126 (B5) and one stout (B6) commercial beers were analyzed.

127 **2.4. Derivatization/extraction conditions**

128 The derivatization and extraction conditions were optimized in model and real wine and beer
129 samples spiked with reference thiols at 5 ng/L. The most suitable conditions were determined
130 by comparing absolute peaks areas. Derivatization/extraction conditions were finally fixed as
131 follows: 20 mL of sample were weighed into a screw cap-tube, added with 400 µL of EDTA 30
132 mg/mL and spiked with 4-methoxy- α -toluenethiol (IS) to a final concentration of 10 ng/L. 6 mL
133 of ebselen 0.1 mM in dichloromethane was then added and the mixture was vortex-mixed
134 during 1 min. The sample was maintained under nitrogen atmosphere during this process.

135 After centrifugation (4000 rpm, 15 min), 1 mL of the dichloromethane phase was collected,
136 dried under nitrogen flow and redissolved in 0.1 mL of methanol.

137 **2.5. High performance liquid chromatography (HPLC)**

138 The HPLC system consisted of a Surveyor MS Plus pump coupled to an Accela Open automatic
139 sampler (Thermo Fisher Scientific, San Jose, California) equipped with 10 μ L loop. The
140 chromatographic separation was performed on a Luna C18 (150 \times 2.1 mm, 5 μ m) analytical
141 column (Phenomenex, Torrance, CA). Elution was performed at a flow rate of 200 μ L/min,
142 using water (solvent A) and methanol (solvent B), both containing ammonium formate 10 mM.
143 The solvent gradient changed according to the following conditions: 50% (A)-50% (B) during 2
144 min, to 100% (B) in 18 min; 100% (B) during 13 min, then to 50% (A)-50% (B) in 1 min,
145 followed by 6 min of maintenance.

146 **2.6. High resolution mass spectrometry (HRMS)**

147 Mass spectrometric analysis was carried out with an Orbitrap-Exactive-HCD (Thermo Fisher
148 Scientific, Bremen, Germany) equipped with an electrospray source (H-ESI II). The ionization
149 conditions in positive mode were according to [Vichi et al. \(2013\)](#): spray voltage 3.75 kV,
150 capillary voltage 25 V, skimmer voltage 16 V, tube lens voltage 80 V. Sheath gas flow rate was
151 set at 40 arbitrary units (au), auxiliary gas flow rate was 10 au, capillary temperature was 275
152 $^{\circ}$ C, and heater temperature was 30 $^{\circ}$ C. The mass range was set to m/z 50-1200. The automatic
153 gain control was used to full fill the C-trap and gain accuracy in mass measurements (high
154 dynamic range mode, 3×10^6 ions). Maximum injection time was 500 ms. High resolving power
155 defined as R: 50,000 (m/z 200, FWHM), 2 HZ, was set. High energy Collision Dissociation (HCD)
156 voltage was fixed at 25 eV. In a single injection, the Orbitrap mass analyzer alternated full scan
157 mode and "All Ion Fragmentation" (AIF) mode at a resolution power of 50,000 (m/z 200,
158 FWHM). Mass accuracies better than 2 ppm were achieved for molecular and product ions,
159 always working with external calibration.

160 The molecular formulae calculation was performed with Xcalibur 2.1 (Thermo Fisher Scientific,
161 Bremen, Germany). In order to obtain a reliable list of confidence formulae from a mass
162 measurement, heuristic filtering (criteria) were set to generate reliable elemental formulae: C
163 ≥ 13 , O ≥ 1 , N=1, Se=1, S=1, and RDB ≥ 8.5 . The mass peaks considered were single positive
164 charged ions $>10^3$ area counts.

165 **2.7. Validation**

166 Quantification was performed using an internal standard and by constructing matrix-matched
167 calibration curves in the range 0.01-50 ng/L, except for 3MBT (5-50 ng/L). Matrix-matched
168 calibration curves were made by analyzing blank wine or beer samples spiked with different
169 amounts of reference thiols dissolved in methanol. Spiked matrix solutions were derivatized
170 and extracted as previously described for samples.

171 Linearity within these ranges was evaluated by the correlation coefficient, r.

172 Experimental limits of quantification (LOQ) were determined by the lowest point of the
173 calibration curve, which was assessed in accordance with the established identification criteria:
174 retention time drift <3 -fold the standard deviation (SD) of the method, mass accuracy < 2 ppm
175 with real resolution ≥ 20.000 (full width at half maximum – FWHM) at the mass range of
176 interest, and the presence of isotope ions containing ^{80}Se and ^{78}Se , respectively.

177 The precision, expressed as intra- and inter-day relative standard deviation (RSD) was obtained
178 analysing the same blank wine and beer spiked with thiols at two concentration levels: 1 and
179 20 ng/L. The repeatability was calculated on seven samples analyzed within the same day. The
180 intra-day RSD was calculated in seven samples analysed on the same day, whereas the inter-
181 day RDS was obtained from six samples analysed on different days.

182 The recovery of the extraction and the matrix effect were assessed by comparing peak areas of
183 reference thiols derivatized with ebselen 0.1 mM in dichloromethane with peak areas of thiols
184 after the derivatization/extraction step, using the same ebselen solution. Thiols were dissolved

185 in 20 mL of: model wine, model beer, white wine, red wine and beer, respectively. Recovery
186 and matrix effect were calculated for eleven reference thiols at two concentration levels: 1 and
187 20 ng/L.

188 **3. RESULTS AND DISCUSSION**

189 As reported by [Xu et al. \(2010\)](#), [Mugesh et al. \(2001\)](#) and [Sarma e al. \(2005\)](#), the reaction
190 between the SH group of the thiol and the Se-N bond of ebselen yields a selenenyl sulfide
191 derivative through the formation of an Se-S bond (**Fig S2, Supplementary information**). This
192 fast, selective and efficient reaction enables the derivatization and isolation of volatile thiols
193 from hydroalcoholic matrixes by a simple liquid–liquid extraction, over a minimum time and
194 with minimum sample manipulation. A selective derivatization strategy is the key to stabilizing
195 the free thiol group. The use of ebselen as the derivatization agent allows the reactive thiol
196 group to be protected and the ESI-HRMS responses of the derivative to be increased due to
197 the presence of the easily ionizable aminic nitrogen atom. Moreover, selective chemical
198 tagging of volatile thiol with a molecule containing selenium allowed outstanding detection
199 selectivity. In fact, after derivatization with ebselen, all the thiols showed the characteristic
200 selenium isotopic pattern, as exemplified by 3MHA extracted from spiked wine (1 ng/L) (**Fig.**
201 **1a**), which fitted the theoretical isotopic pattern perfectly (**Fig. 1b**). Isotope M+6, which
202 matches the presence of an ^{80}Se atom, presented the strongest signal; followed by M+4, which
203 corresponds to the presence of ^{78}Se . Isotopes M+6 and M+4 were used as quantification and
204 confirmation ions, respectively.

205 Compliance with the identification criteria for derivatized thiols are reported in **Table 1**.
206 Independently of the concentration and matrix tested, mass accuracy was always better than 2
207 ppm, with a SD of the mass error of between 0.1 and 0.4 ppm. The real resolution was >
208 28,000 for all the thiols analyzed.

209 **3.1.1. Optimization of derivatization/extraction conditions**

210 The derivatization and extraction conditions previously optimized for lipid samples (Vichi et al.,
211 2013) were adapted to hydroalcoholic samples by using dichloromethane as the extraction
212 solvent. Distinct solvent volumes and ebselen concentrations were tested to optimize the
213 recovery of derivatized thiols in such matrixes. The sample volume to extraction solvent
214 volume ratio was proportional to the thiol uptake (Fig. S3a, supplementary information). Given
215 that above ratios of 2.5-3 the increase in thiol uptake was low, higher volume ratios were not
216 tested. To enable easy collection of a discrete volume of clean sample extract after
217 centrifugation, the final sample volume to dichloromethane volume ratio was fixed at 20:6 mL.
218 No substantial differences were observed in the thiol response using ebselen concentrations of
219 between 0.05 and 0.1 mM for the analysis of beer or wine spiked at 10 ng/L (Fig. S3b,
220 supplementary material). However, the highest of these ebselen concentrations was chosen
221 for further analysis to ensure complete derivatization of thiols even at the higher
222 concentrations reported in the literature (Tominaga et al., 2000; Vermuelen et al., 2006).
223 Prior to the analysis, a concentration step was applied in order to increase the response of the
224 thiols, and dichloromethane was replaced by methanol to improve the chromatography.
225 Representative thiols containing different functional groups were chosen to develop and
226 evaluate the method. They expressly included primary, secondary and tertiary thiols in order
227 to detect any discrimination effect in their derivatization. The exact mass, the molecular
228 formula and the molecular structure of the selected reference thiols are shown in Table 1 and
229 Fig. S1 (Supplementary information).

230 3.2. LC-ESI-HRMS analysis

231 The chromatograms obtained from the derivatized extract of a white wine spiked with
232 reference thiols at 1 ng/L (IS 10 ng/L; 3MTB 20 ng/L), by selecting the exact mass of each thiol
233 derivative from the ESI+ full-scan analysis, are shown in Fig. S4 (Supplementary information).
234 The chromatographic retention time drifts, expressed as SD and calculated from samples

235 analyzed in different days, are reported in **Table 1**. As can be observed, HRMS provided high
236 selectivity and sensibility, with almost no noise. HRMS was chosen since by means of accurate
237 mass (AM) measured with high resolving power it provides the best information about the
238 molecular composition of the compounds, thereby allowing us to confirm or tentatively
239 identify their elemental formulae. Moreover, it has been demonstrated in several studies that
240 high resolution is necessary in the analysis of complex samples in order to avoid interference
241 from isobaric compounds and thus the problem of false positives ([Kaufmann, 2012](#)).

242 **3.3. Validation.**

243 **3.3.1. Recovery and matrix effect**

244 In order to estimate the effect of the matrix on the extraction of derivatized thiols, their
245 recovery was assessed in spiked red and white wine, beer, model wine and model beer. The
246 response of thiols after derivatization and extraction in such matrixes was compared with the
247 response obtained by adding the same concentrations of thiols directly to the ebselen
248 solution. **Table 1** reports the recovery of the thiols from each matrix, calculated as the
249 percentage of the peak areas. The extraction of derivatized thiols, evaluated at two
250 concentration levels, can be considered as quantitative in model systems, beer and white wine
251 for all the thiols except 3MBT, which in some cases presented slightly lower recoveries (though
252 always over 45%). In the case of red wine, a moderate, overall decrease of thiol recovery was
253 observed.

254 **3.3.2. Method sensitivity and linearity**

255 Quantification was carried out using an internal standard and by constructing matrix-matched
256 calibration curves using white wine and beer spiked at the concentrations reported in **Table 2**.
257 The lower limit of these ranges was determined by the limit of quantification (LOQ) of each
258 thiol. Linearity within these ranges, evaluated by the correlation coefficient (r) was > 0.98 ,
259 except for 3MBT in wine ($r = 0.9779$).

260 The experimental LOQ was given by the lowest concentration which it was possible to measure
261 according to the established identification criteria: retention time drift, mass accuracy,
262 resolution, and the presence of ions containing the isotopes ⁸⁰Se and ⁷⁸Se, as detailed in the
263 Material and methods section.

264 The LOQ of the eleven reference thiols, assessed in wine and beer matrixes, ranged from 0.01
265 to 5 and from 0.01 to 10 ng/L, respectively (**Table 2**): slightly higher in beer than in wine. These
266 LOQ values were compared with those obtained by other analytical methods, when available.
267 The LOQ for 3MH and 3MHA in wine (0.01 ng/L) were far below those obtained by other
268 methods: 0.83 and 4.3 ng/L (Rodríguez-Bencomo et al., 2009); 1 and 0.7-5 ng/L (Schneider et
269 al. 2003), 3.1 ng/L (Sarrazin, Shinkaruk, Tominaga, Bennetau, Frérot & Dubordieu , 2007), 20
270 and 1.9 ng/L (Mateo-Vivaracho et al, 2007); 0.8 and 6 ng/L (Mateo-Vivaracho, 2008). Likewise,
271 the LOQ for 2FMT in white wine (0.01 ng/L) was lower than previous values: 1.4 ng/L (Mateo-
272 Vivaracho et al, 2007), 0.3 ng/L (Mateo-Vivaracho, 2008), 2.2 ng/L (Tominaga & Dubourdieu,
273 2006) and below the reported odor threshold: 0.4 ng/L (Tominaga et al., 2000).

274 3MBT showed a LOQ higher than those of the other thiols. As standard 3MBT is not easily
275 available, a commercial preparation kit was used to obtain this compound, for which the
276 reaction yield could not be verified. A low reaction yield during standard preparation would
277 explain the higher LOQ calculated for this compound. As far as we know, no LOQ data are
278 available for 3MBT by other methods, but an odor threshold of from 1 to 35 ng/L was reported
279 for this thiol (Hugues, 2009). Overall, the thiol LOQ are lower than their odor thresholds
280 reported in the literature (Tominaga et al., 2000; Mestres et al., 2000; Hugues, 2009),
281 indicating that the present method is suitable for current purposes.

282 **3.3.3. Method precision**

283 The precision of the method, expressed as intra-day and inter-day relative standard deviation
284 (RSD), was calculated at two concentration levels fixed in the low and mid-range of the

285 calibration curve and in two matrixes: wine and beer (**Table 2**). Intra-day RSD was in general
286 below 10%, while inter-day RSD ranged between 5% and 30%.

287 **3.4. Non-target analysis**

288 Prompt fragmentation corresponding to the S-Se bond indicated in the scheme (**Fig. 1c**) means
289 that thiol structural information cannot be obtained by MS/MS analysis. However, the
290 formation of a diagnostic product ion at m/z 275.9922 [$C_{13}H_{10}ONSe$]⁺, which corresponds to
291 the ebselen moiety of the derivatives and preserves the typical selenium isotopic pattern, is
292 useful for the screening of non-target thiols via full-scan mode acquisition, with and without
293 HCD fragmentation. The presence of a non-target derivatized thiol may be revealed by the
294 presence of the diagnostic ion in the HCD chromatogram. Thiol identification must be
295 confirmed by the presence in the full-scan chromatogram of the corresponding identification
296 and confirmation ions (isotopes M+6 and M+4) that fit with the restrictions established for
297 their molecular composition.

298 **3.5. Analysis of wine and beer samples**

299 In order to evaluate the suitability of the optimized method for the analysis of real
300 hydroalcoholic beverages, four white wines and six beers were selected for analysis. Five out
301 of the eleven reference thiols studied were identified and quantified in the samples analyzed
302 (**Table 3**). In the same samples, fourteen non-target thiols were detected and quantified by
303 expressing their concentration as ng IS equivalent/L. **Table 3** shows the exact mass and the
304 elemental composition of these thiol derivatives. Some of them were also tentatively
305 identified on the basis of their molecular formula and reports of their occurrence in wine or
306 beer, when available. Both target and non-target thiols were characterized by the diagnostic
307 ion m/z 275.9922, according to the restrictions established for their molecular composition,
308 and contained both quantification and confirmation ions (⁸⁰Se and ⁷⁸Se, respectively),
309 identified with a mass accuracy < 2 ppm at R > 28,000.

310 The target thiols 3MH and 3MHA were present in wines at concentrations of from 0.21 to 1.32
311 ng/L and from 0.01 to 0.22 ng/L, respectively; they were most abundant in Sauvignon Blanc, as
312 expected. 3MMB was found in both wine and beer samples, in the range 0.01-0.03 ng/L. 3MBT
313 reached 37.3 and 3.4 ng/L in lager beer samples exposed to light, while it was not detected in
314 other samples. Finally, 2FMT was identified in the stout beer sample, probably due to the use
315 of roasted malt or barely in the production of this product. Anyway, it was at a concentration
316 below the LOQ (<0.1 ng/L).

317 Regarding non-target thiols, those tentatively identified as methyl mercaptopropionate, ethyl
318 mercaptopropionate, methanethiol and mercaptoethanol were the most abundant in wine
319 samples and stout beer, in some cases reaching concentrations of nearly 50 ng/L (expressed as
320 equivalents of IS). Methanethiol was the most abundant non-target thiol in lager beers,
321 ranging between 1.9 and 4.6 ng/L.

322 The importance of HRMS for the correct identification of compounds is demonstrated in **Fig. 2**,
323 which shows the presence in a wine sample of two derivatized thiols with the same nominal
324 mass and quite similar chromatographic retention times, but different molecular formula: the
325 tentatively identified ethyl 3-mercaptopropionate ($C_{18}H_{20}O_3NSSe$, m/z 410.0324) (**Fig. 2a**) and
326 3MH ($C_{19}H_{24}O_2SSe$, m/z 410.0687) (**Fig. 2b**). The same figure shows the isotopic patterns of
327 both derivatized thiols, and their compliance with the identification criteria.

328

329 In conclusion, the simultaneous derivatization/extraction method followed by ESI-LC-HRMS
330 was optimized for the determination of volatile thiols in hydroalcoholic matrixes and used to
331 identify and quantify volatile thiols in real wine and beer samples. The method was shown to
332 be fit for this purpose by carrying out a validation study to ensure reliable results.
333 Experimental LOQs were between 0.01 and 0.05 ng/L for most of the thiols evaluated, and
334 lower than those available in the literature. Acceptable recoveries were obtained in model

335 and real wine and beer matrixes, as well as satisfactory intra-day and inter-day RSD values. Any
336 positive finding had to satisfy the identification criteria established, based on retention time
337 drift, mass accuracy, real resolution, and the presence of identification and confirmation ions.
338 Five target thiols were identified and quantified in wine and beer samples, while fourteen thiol
339 derivatives were detected by the non-target approach, which were tentatively identified on
340 the basis of their molecular formula.

341

342 **Acknowledgements**

343 This study was supported by the Spanish *Ministerio de Economía y Competitividad* through the
344 Ramón y Cajal program (RYC-2010-07228) and the project AGL 2011-23872.

345

346 **Literature cited**

- 347 Blanchard, L., Tominaga, T. & Dubourdieu, D. (2001) Formation of Furfurylthiol Exhibiting a
348 Strong Coffee Aroma during Oak Barrel Fermentation from Furfural Released by Toasted
349 Staves. *Journal of Agricultural and Food Chemistry*, 49, 4833-4835.
- 350 Bouchilloux, P., Darriet, P., Henry, R., Lavigne-Cruege, V. & Dubourdieu, D. (1998) Identification
351 of Volatile and Powerful Odorous Thiols in Bordeaux Red Wine Varieties. *Journal of*
352 *Agricultural and Food Chemistry*, 46, 3095-3099.
- 353 Cosser, K.B. , Murray, J.P. & Holzapfel, C.W. (1980) Investigation of a ribes off-flavor in beer.
354 *Technical Quarterly MBAA Communication*, 17, 53-59.
- 355 Darriet, P., Tominaga, T., Lavigne, V., Boidron, J.N. & Dubourdieu D. (1995) Identification of a
356 powerful aromatic component of *Vitis vinifera* L. var. sauvignon wines: 4-mercapto-4-
357 methylpentan-2-one. *Flavour and Fragrance Journal*, 10, 385-392.
- 358 Eagles, W.P. & Wakeman, R.J. (2002) Interactions between dissolved material and the fouling
359 layer during microfiltration of a model beer solution. *Journal of Membrane Science*, 206,
360 253-264.
- 361 Goldstein, H., Rader, S. & Murakami, A. (1993) Determination of 3-methyl-2-butene-1-thiol in
362 beer. *Journal of the American Society of Brewing Chemists*, 51, 70-74.
- 363 Guth, H. (1997) Quantitation and sensory studies of character impact odorants of different
364 white wine varieties. *Journal of Agricultural and Food Chemistry*, 45, 3027-3032.
- 365 Hugues, P. in: Russell, I., Bamforth, C. W. & Stewart G. (Eds.), Beer, a quality perspective-
366 Handbook of Alcoholic Beverages, Academic press, London, 2009, pp 61.
- 367 Huvaere, K., Andersen, M.L., Skibsted, L.H., Heyerick, A. & Keukeleire, D. (2005)
368 Photooxidative Degradation of Beer Bittering Principles: A Key Step on the Route to
369 Lightstruck Flavor Formation in Beer. *Journal of Agricultural and Food Chemistry*, 53,
370 1489-1494.

371 Kaufmann, A. (2012) The current role of high-resolution mass spectrometry in food analysis.
372 *Analytical and Bioanalytical Chemistry*, 403, 1233-1249.

373 Kotseridis, Y. & Baumes, R. (2000) Identification of Impact Odorants in Bordeaux Red Grape
374 Juice, in the Commercial Yeast Used for Its Fermentation, and in the Produced Wine.
375 *Journal of Agricultural and Food Chemistry*, 48, 400-406.

376 Labanda, J., Vichi, S., Llorens, J. & López-Tamames, E. (2009) Membrane separation technology
377 for the reduction of alcoholic degree of a white model wine. *LWT - Food Science and*
378 *Technology*, 42, 1390-1395.

379 Lermusieau, G., Bulens, M. & Collin, S. (2001) Use of GC-Olfactometry to Identify the Hop
380 Aromatic Compounds in Beer J. *Journal of Agricultural and Food Chemistry*, 49, 3867-
381 3874.

382 Lermusieau, G. & Collin, S. (2003) Volatile sulfur compounds in hops and residual
383 concentrations in beer-A review. *Journal of the American Society of Brewing Chemists*, 61,
384 109-113.

385 McGorrin, R.J. in: M. Quian et al. (Eds.), Volatile sulfur compounds in foods, ACS Symposium
386 Series, American Chemical Society, Washington, 2011, pp.3.

387 Mateo-Vivaracho, L., Cacho, J. & Ferreira, V. (2007) Quantitative determination of wine
388 polyfunctional mercaptans at nanogram per liter level by gas chromatography–negative
389 ion mass spectrometric analysis of their pentafluorobenzyl derivatives. *Journal of*
390 *Chromatography A*, 1146, 242-250.

391 Mateo-Vivaracho, L., Cacho, J. & Ferreira, V. (2008) Improved solid-phase extraction procedure
392 for the isolation and in-sorbent pentafluorobenzyl alkylation of polyfunctional
393 mercaptans: Optimized procedure and analytical applications. *Journal of Chromatography*
394 *A*, 1185, 9-18.

395 Mestres, M., Busto, O. & Guasch, J. (2000) Analysis of organic sulfur compounds in wine aroma.
396 *Journal of Chromatography A*, 881, 569-581.

397 Mugesh, G., du Mont, W.W. & Sies, H. (2001) Chemistry of Biologically Important Synthetic
398 Organoselenium Compounds. *Chemical Reviews*, 101, 2125-2180.

399 Rodríguez-Bencomo, J.J., Schneider, R. , Lepoutre, J.P. & Rigou, P. (2009) Improved method to
400 quantitatively determine powerful odorant volatile thiols in wine by headspace solid-
401 phase microextraction after derivatization. *Journal of Chromatography A*, 1216, 5640-
402 5646.

403 Sarma, B.K. & Mugesh, G. (20025) Glutathione Peroxidase (GPx)-like Antioxidant Activity of the
404 Organoselenium Drug Ebselen: Unexpected Complications with Thiol Exchange Reactions.
405 *Journal of the American Chemists Society*, 127, 11477-11485.

406 Sarrazin, E., Shinkaruk, S., Tominaga, T., Benneteau, B., Frérot, E. & Dubordieu D. (2007)
407 Odorous Impact of Volatile Thiols on the Aroma of Young Botrytized Sweet Wines:
408 Identification and Quantification of New Sulfanyl Alcohols. *Journal of Agricultural and*
409 *Food Chemistry*, 55, 1437-1444.

410 Schieberle, P. (1991) Primary odorants of pale lager beer. *Zeitschrift für Lebensmittel*
411 *Untersuchung und Forschung A*, 193, 558-565.

412 Schneider, R., Charrier, F., Razungles & A. Baumes, R. (2006) Evidence for an alternative
413 biogenetic pathway leading to 3-mercaptohexanol and 4-mercapto-4-methylpentan-2-
414 one in wines. *Analytica Chimica Acta*, 536, 58-64.

415 Schneider, R. , Kotseridis, Y., Ray, J.L., Augier, C. & Baumes R. (2003) Quantitative determination
416 of sulfur-containing wine odorants at sub parts per billion levels. 2. Development and
417 application of a stable isotope dilution assay. *Journal of Agricultural and Food Chemistry*,
418 51, 3243-3248.

419 Tominaga, T., Baltenweck-Guyot, R., Peyrot des Gachons, C. & Dubourdieu, D. (2000)
420 Contribution of Volatile Thiols to the Aromas of White Wines Made From Several *Vitis*
421 *vinifera* Grape Varieties. *American Journal of Enology and Viticulture*, 51, 178-181.

422 Tominaga, T., Blanchard, L., Darriet, P. & Dubourdieu, D. (2000) A Powerful Aromatic Volatile
423 Thiol, 2-Furanmethanethiol, Exhibiting Roast Coffee Aroma in Wines Made from
424 Several *Vitis vinifera* Grape Varieties. *Journal of Agricultural and Food Chemistry*, 48,
425 1799-1802.

426 Tominaga, T. & Dubourdieu, D. (2006) A novel method for quantification of 2-methyl-3-
427 furanthiol and 2-furanmethanethiol in wines made from *Vitis vinifera* grape varieties. J.
428 *Journal of Agricultural and Food Chemistry*, 54, 29-33.

429 Tominaga, T., Guimbertau, G. & Dubourdieu, D. (2003) Role of Certain Volatile Thiols in the
430 Bouquet of Aged Champagne Wines. *Journal of Agricultural and Food Chemistry*, 51,
431 1016-1020.

432 Tominaga, T., Murat, M. & D. Dubourdieu (1998) Development of a Method for Analyzing the
433 Volatile Thiols Involved in the Characteristic Aroma of Wines Made from *Vitis vinifera* L.
434 Cv. Sauvignon Blanc. *Journal of Agricultural and Food Chemistry*, 46, 1044-1048.

435 Vermeulen, C., Gijs, L. & Collin, S. (2005) Sensorial Contribution and Formation Pathways of
436 Thiols in Foods: A Review. *Food Reviews International*, 21, 69-137.

437 Vermeulen, C., Lejeune, I., Tran, T.T.H. & Collin, S. (2006) Occurrence of Polyfunctional Thiols in
438 Fresh Lager Beers *Journal of Agricultural and Food Chemistry*, 54, 5061-5068.

439 Vichi, S., Cortés-Francisco, N. & Caixach, (2013) J. Determination of volatile thiols in lipid matrix
440 by simultaneous derivatization/extraction and liquid chromatography–high resolution
441 mass spectrometric analysis. Application to virgin olive oil. *Journal of Chromatography A*,
442 1318,180-188.

443 Xu, Y., Zhang, B., Tang, J., Laskin, P. J., Roach, H. & Chen, H. (2010) Study of Highly Selective and
444 Efficient Thiol Derivatization Using Selenium Reagents by Mass Spectrometry. *Analytical*
445 *Chemistry*, 82, 6926-6932.
446

447 **Figure legends**

448 **Figure 1.** (a) Mass spectrum of 3MHA, $[\text{C}_{21}\text{H}_{26}\text{O}_3\text{NSSe}]^+$, at 1 ng/L, derivatized and extracted
449 from wine; (b) theoretical isotopic pattern of $[\text{C}_{21}\text{H}_{26}\text{O}_3\text{NSSe}]^+$; (c) mass spectrum and
450 molecular structure of diagnostic ion $[\text{C}_{13}\text{H}_{10}\text{ONSe}]^+$, m/z 275.9922; R: 50,000 (m/z 200,
451 FWHM), mass error tolerance < 2ppm; HCD voltage: 25 eV.

452 **Figure 2.** ESI+ Full scan chromatogram obtained by monitoring the exact mass of a) tentatively
453 identified ethyl 3-mercaptopropionate-ethylselen derivative at m/z 410.0324, and b) 3MH-
454 ethylselen derivative at m/z 410.0687, in wine sample W2; both with the corresponding mass
455 spectrum showing identification and confirmation ions: isotopes M+6 and M+4, relative to the
456 presence of isotopes ^{80}Se and ^{78}Se , respectively. R: 50,000 (m/z 200, FWHM), mass error
457 tolerance < 2ppm.

458

TABLES

Table 1. Molecular formulae, exact mass, mass accuracy and precision values, real mass resolution, chromatographic retention time and precision values for ebselen-derivatized thiols in positive ESI, setting the R: 50,000 (m/z 200, FWHM); recovery (%) of the extraction and standard deviation (SD) calculated real and model wine and beer matrices and at two concentration levels, by comparison with derivatized thiols in dichloromethane solution ($n=3$). Peak numbering is according to **Fig. 2** and **S1 (supplementary information)**.

Compound	Formula [M+H] ⁺	Theoretical m/z	Δ^a (ppm) \pm SD	RT ^c (min) \pm SD	Recovery (%) \pm SD										
					1 ng/L					20 ng/L					
					model beer ($n=3$)	beer ($n=3$)	model wine ($n=3$)	white wine ($n=3$)	red wine ($n=3$)	model beer ($n=3$)	beer ($n=3$)	model wine ($n=3$)	white wine ($n=3$)	red wine ($n=3$)	
1	3MMB-Ebs	C ₁₈ H ₂₂ O ₂ NSSe	396.0531	1.5 \pm 0.3	14.34 \pm 0.05	102 \pm 14	80 \pm 14	99 \pm 14	79 \pm 17	59 \pm 5	91 \pm 7	73 \pm 13	98 \pm 14	75 \pm 9	61 \pm 9
2	4MMP-Ebs	C ₁₉ H ₂₄ O ₂ NSSe	410.0687	1.7 \pm 0.3	15.38 \pm 0.04	121 \pm 3	91 \pm 16	104 \pm 23	105 \pm 18	75 \pm 8	120 \pm 13	98 \pm 15	113 \pm 13	107 \pm 11	91 \pm 13
3	3MH-Ebs	C ₁₉ H ₂₄ O ₂ NSSe	410.0687	1.6 \pm 0.3	16.35 \pm 0.04	129 \pm 10	74 \pm 11	110 \pm 19	107 \pm 22	84 \pm 9	121 \pm 12	81 \pm 12	115 \pm 17	97 \pm 10	80 \pm 7
4	4MMB-Ebs	C ₁₉ H ₂₄ O ₂ NSSe	410.0687	1.4 \pm 0.2	17.45 \pm 0.04	114 \pm 12	70 \pm 21	108 \pm 14	93 \pm 16	73 \pm 12	120 \pm 14	95 \pm 18	110 \pm 16	100 \pm 12	86 \pm 15
5	2FMT-Ebs	C ₁₈ H ₁₆ O ₂ NSSe	390.0061	1.3 \pm 0.1	16.82 \pm 0.02	121 \pm 16	83 \pm 15	103 \pm 12	103 \pm 14	83 \pm 11	119 \pm 15	86 \pm 12	100 \pm 6	92 \pm 9	86 \pm 18
	IS	C ₂₁ H ₂₀ O ₂ NSSe	430.0374	1.3 \pm 0.2	18.19 \pm 0.02	119 \pm 9	84 \pm 16	104 \pm 23	99 \pm 17	66 \pm 13	127 \pm 14	102 \pm 30	120 \pm 20	101 \pm 13	85 \pm 14
6	3MHA-Ebs	C ₂₁ H ₂₆ O ₃ NSSe	452.0793	1.5 \pm 0.2	18.83 \pm 0.02	123 \pm 11	94 \pm 25	112 \pm 18	103 \pm 21	80 \pm 17	111 \pm 15	102 \pm 24	107 \pm 14	98 \pm 14	79 \pm 13
7	3MBT-Ebs	C ₁₈ H ₂₀ ONSSe	378.0419	1.6 \pm 0.2	18.96 \pm 0.04	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	71 \pm 21	74 \pm 15	70 \pm 15	46 \pm 11	45 \pm 22
8	1PEM-Ebs	C ₂₁ H ₂₀ ONSSe	414.0425	1.5 \pm 0.2	19.11 \pm 0.03	109 \pm 9	84 \pm 18	103 \pm 26	96 \pm 15	72 \pm 14	107 \pm 18	97 \pm 16	101 \pm 14	92 \pm 12	80 \pm 12
9	MT-Ebs ^d	C ₂₃ H ₂₈ O ₂ NSSe	462.1000	1.6 \pm 0.2	19.32 \pm 0.03	118 \pm 12	90 \pm 22	107 \pm 18	101 \pm 23	80 \pm 17	121 \pm 13	109 \pm 27	113 \pm 18	102 \pm 17	84 \pm 15
10	HT-Ebs	C ₁₉ H ₂₄ ONSSe	394.0738	1.5 \pm 0.3	21.44 \pm 0.02	89 \pm 12	91 \pm 24	94 \pm 25	79 \pm 16	66 \pm 15	99 \pm 24	112 \pm 28	89 \pm 15	91 \pm 13	87 \pm 15
11	3MHH-Ebs	C ₂₅ H ₃₄ O ₃ NSSe	508.1419	1.2 \pm 0.3	22.12 \pm 0.02	104 \pm 10	87 \pm 25	101 \pm 18	94 \pm 19	78 \pm 17	98 \pm 10	106 \pm 31	106 \pm 17	100 \pm 15	88 \pm 11

^a: mean of $n=6$ replicates analysed in different days for blank beer and wine spiked samples considered together, expressed as root mean square error (RMS error) \pm standard deviation (SD), both in ppm; ^b: R: real mass resolution; ^c: chromatographic retention time \pm SD ($n=14$); ^d: MT, mixture of isomers

Table 2. Linearity range calculated in white wine and beer matrix, evaluated by regression coefficient (r); experimental limit of quantification (LOQ) consisting in the lowest concentration satisfying the established confirmation criteria; method repeatability and reproducibility, calculated at two concentration levels, and expressed as intra-day relative standard deviation (RSD) and inter-day RSD, respectively.

Compound	Wine								Beer							
	Range (ng/L)	Linearity (r)	LOQ (ng/L)	Intra-day RSD (%) (n=7)		Inter-day RSD (%) (n=6)		Range (ng/L)	Linearity (r)	LOQ (ng/L)	Intra-day RSD (%) (n=7)		Inter-day RSD (%) (n=6)			
				1 ng/L	20 ng/L	1 ng/L	20 ng/L				1 ng/L	20 ng/L	1 ng/L	20 ng/L		
1 3MMB-Ebs	0.01-50	0.9942	0.01	7	6	18	12	0.01-50	0.9956	0.01	5	4	13	15		
2 4MMP-Ebs	0.01-50	0.9896	0.01	4	7	20	10	0.05-50	0.9916	0.05	5	4	10	11		
3 3MH-Ebs	0.01-50	0.9961	0.01	5	6	19	12	0.05-50	0.9962	0.05	6	6	16	17		
4 4MMB-Ebs	0.01-50	0.9949	0.01	4	9	19	15	0.05-50	0.9856	0.05	6	5	9	6		
5 2FMT-Ebs	0.01-50	0.9902	0.01	4	9	12	18	0.1-50	0.9803	0.1	8	6	16	12		
6 3MHA-Ebs	0.01-50	0.9955	0.01	8	7	17	11	0.05-50	0.9932	0.05	8	4	8	9		
7 3MBT-Ebs	5-50	0.9779	5	-	38	-	52	10-50	0.9937	10	-	7	-	26		
8 1PEM-Ebs	0.01-50	0.9865	0.01	9	8	24	17	0.05-50	0.9828	0.05	10	3	11	9		
9 MT-Ebs ^a	0.01-50	0.9964	0.01	5	8	18	11	0.05-50	0.9944	0.05	7	5	14	10		
10 HT-Ebs	0.01-50	0.9942	0.01	6	23	23	30	0.05-50	0.9973	0.05	14	6	14	12		
11 3MHH-Ebs	0.01-50	0.9897	0.01	7	9	22	15	0.05-50	0.9831	0.05	10	6	13	5		

^a:MT, sum of isomers

Table 3. Target (in bold) and non-target volatile thiols detected in selected wine and beer samples.

RT ^c (min)	Theoretical <i>m/z</i>	derivative formula [M+H] ⁺	thiol formula	Identification or tentative identification	Thiols in wine samples (ng/L) ^a				Thiols in beer samples (ng/L) ^b					
					W1	W2	W3	W4	B1	B2	B3	B4	B5	B6
11.43	354.0061	C ₁₅ H ₁₆ O ₂ NSSe	C ₂ H ₆ OS	mercaptoethanol ^d	1.2	4.3	6.9	1.9	0.52	0.33	1.0	0.47	0.97	2.27
11.52	366.0061	C ₁₆ H ₁₆ O ₂ NSSe	C ₃ H ₆ OS		0.10	0.05	0.03	0.17						
11.56	351.9905	C ₁₅ H ₁₄ O ₂ NSSe	C ₂ H ₄ OS	mercaptoacetaldehyde ^d	0.35	0.19	0.13	0.57	0.10	0.22	0.07	0.09	0.09	0.30
12.33	368.0218	C ₁₆ H ₁₈ O ₂ NSSe	C ₃ H ₈ OS	mercaptopropanol ^d	0.37	1.42	1.29	0.24	0.06		0.12		0.05	0.30
12.76	426.0273	C ₁₈ H ₂₀ O ₄ NSSe	C ₅ H ₁₀ O ₃ S	2-hydroxyethyl-3-mercaptopropionate ^d	0.83	0.55	0.80	0.69						
13.02	436.0116	C ₁₉ H ₁₈ O ₄ NSSe	C ₆ H ₈ O ₃ S						0.06		0.08	0.04	0.04	
13.78	382.0374	C ₁₇ H ₂₀ O ₂ SSe	C ₄ H ₁₀ OS	Mercaptobutanol or mercaptomethylpropanol ^d	0.34	1.68	1.00	0.44	0.02		0.04		0.03	0.01
14.36	396.0531	C ₁₈ H ₂₂ O ₂ NSSe	C ₅ H ₁₂ OS	3MMB ^e		0.03	0.01	0.02	0.16	0.20	0.21	0.08	0.13	0.23
14.51	396.0167	C ₁₇ H ₁₈ O ₃ NSSe	C ₄ H ₈ O ₂ S	methyl-3-mercaptopropionate ^d	6.2	31.3	47.1	15.5	1.9	0.53	4.5	1.5	2.0	8.2
14.92	323.9956	C ₁₄ H ₁₄ ONSSe	CH ₄ S	methanethiol ^d	2.9	6.6	3.0	1.6	4.6	1.9	3.4	3.2	2.0	2.7
15.25	410.0324	C ₁₈ H ₂₀ O ₃ NSSe	C ₅ H ₁₀ O ₂ S	ethyl 3-mercaptopropionate ^d	1.1	5.4	6.6	1.5	0.23	0.05	1.02	0.15	0.13	1.02
15.66	408.0531	C ₁₉ H ₂₂ O ₂ SSe	C ₆ H ₁₂ OS	4-mercapto-4-methylpentan-2-one ^d		0.10								
15.8	452.0793	C ₂₁ H ₂₆ O ₃ NSSe	C ₈ H ₁₆ O ₂ S	mercaptohexyl acetate isomer ^d		0.05	0.02	0.01						
16.37	410.0687	C ₁₉ H ₂₄ O ₂ SSe	C ₆ H ₁₄ OS	3MH ^e	0.33	1.3	0.21	0.33						
16.60	338.0112	C ₁₅ H ₁₆ ONSSe	C ₂ H ₆ S	ethanethiol ^d	0.22	0.48	0.56	0.02	0.01		0.08			
16.83	390.0061	C ₁₈ H ₁₆ O ₂ NSSe	C ₅ H ₆ OS	2MFT ^e										<LOQ
16.88	424.0480	C ₁₉ H ₂₂ O ₃ NSSe	C ₆ H ₁₂ O ₂ S	ethyl 3-mercaptobutyrate/mercaptohexanoic acid ^d	0.03	0.12	0.20	0.04		0.04				
18.89	452.0793	C ₂₁ H ₂₆ O ₃ NSSe	C ₈ H ₁₆ O ₂ S	3MHA ^e	0.01	0.22		0.02						
18.91	378.0419	C ₁₈ H ₂₀ ONSSe	C ₅ H ₇ S	3MBT ^e					37.3	3.4				

^a: W1: albariño, W2: sauvignon blanc, W3: Riesling, W4: sauvignon blanc/gewürtztraminer; ^b: B1: lager beer exposed to light, B2: lager beer exposed to light; B3: lager beer3; B4: double malt beer; B5: alcohol free beer, B6: stout beer; ^c: retention time; ^d: tentative identification on the basis of molecular formula; quantified as ng/L of IS; ^e: identified by comparison with authentic reference compound by using matrix-matched calibration curve.

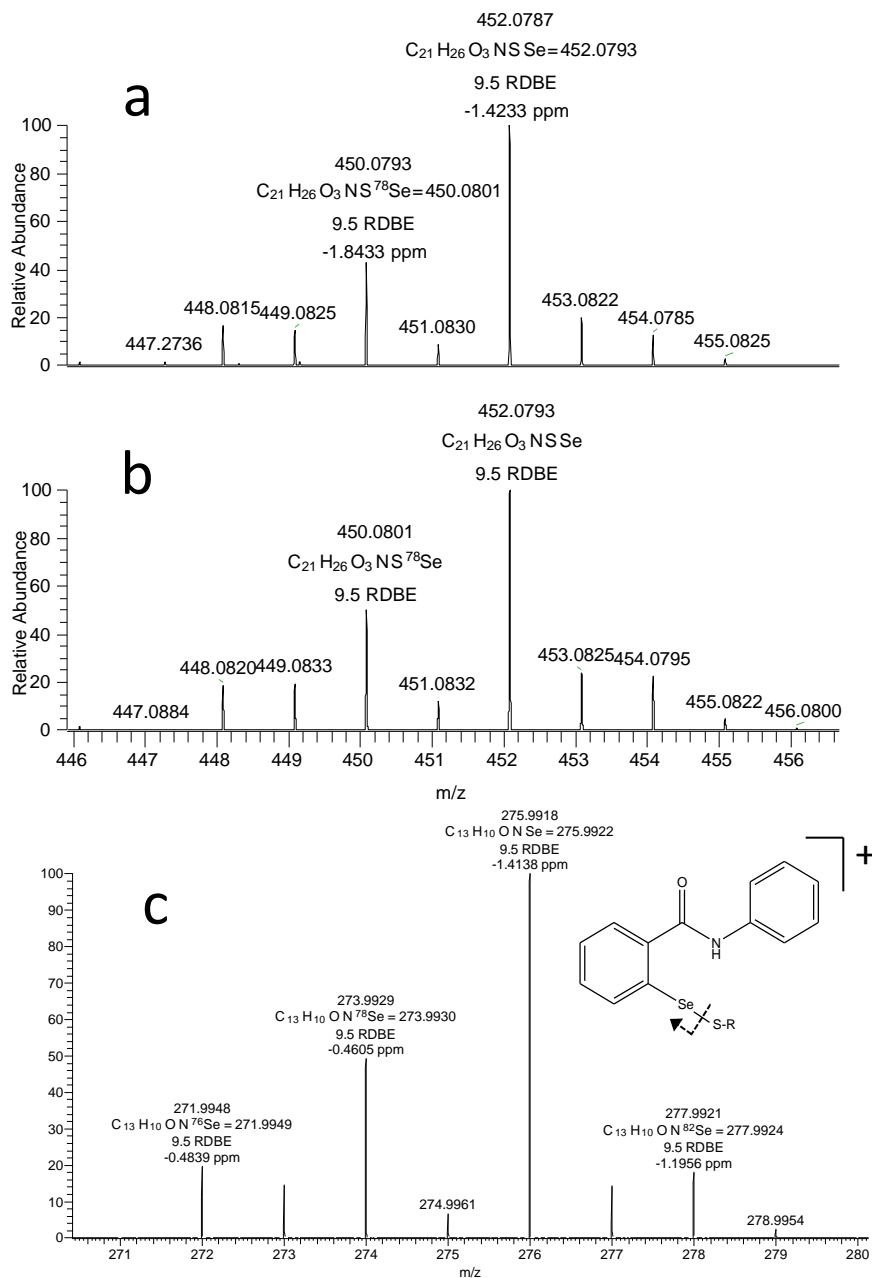


Fig. 1

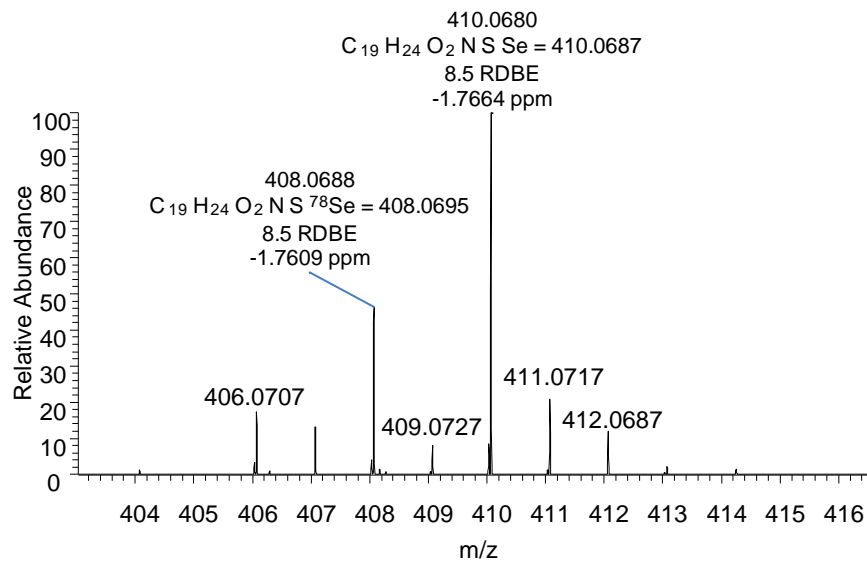
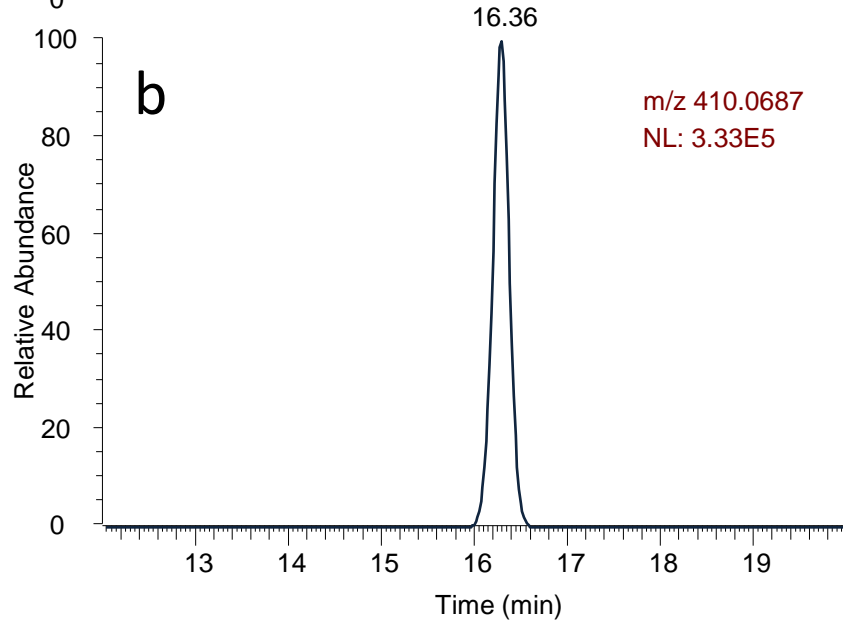
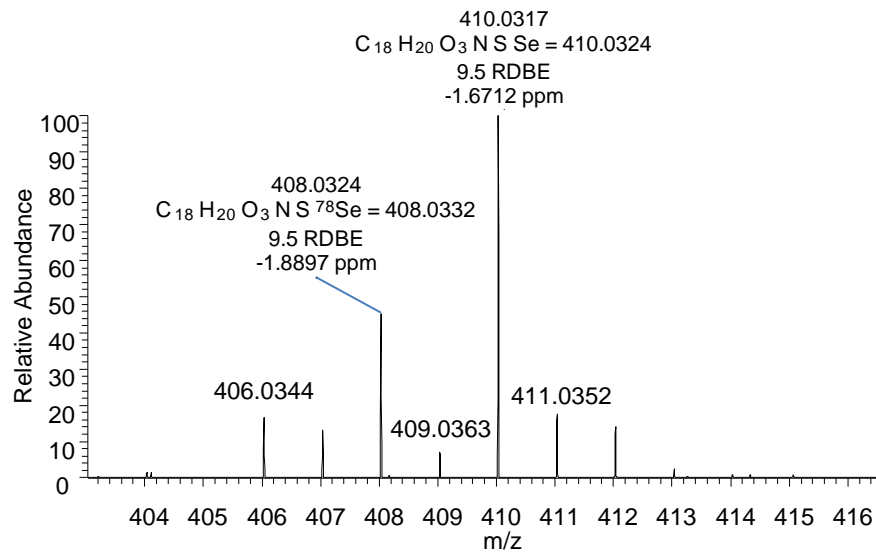
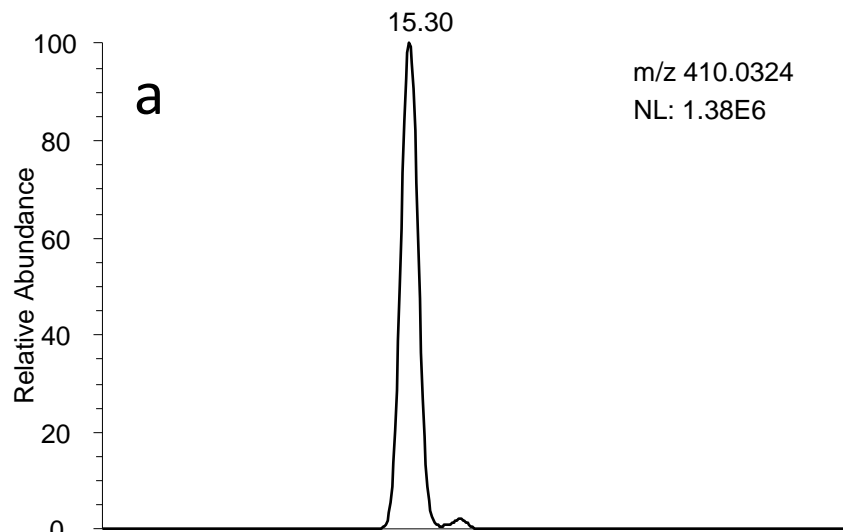


Fig. 3