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Simultaneous extraction, derivatisation and analysis of varietal thiols and their non-volatile precursors from beer

Rebecca E. Jelley [a,*], Hayden Jones-Moore [a], Angela Guan [a], Chloe Z.-J. Ren [b,c], Jack L.-Y. Chen [b,c,d], Loris Tonidandel [e], Roberto Larcher [e], Bruno Fedrizzi [a,f,**]

[a] School of Chemical Sciences, University of Auckland, 23 Symonds St, Auckland, New Zealand
[b] Centre for Biomedical and Chemical Sciences, School of Science, Auckland University of Technology, Auckland, 1010, New Zealand
[c] The MacDiarmid Institute for Advanced Materials and Nanotechnology, Wellington, 6011, New Zealand
[d] Department of Biotechnology, Chemistry and Pharmaceutical Sciences, Università degli Studi di Siena, Via Aldo Moro, 53100, Siena, Italy
[e] Technology Transfer Centre, Fondazione Edmund Mach, Via E. Mach 1, 38010, San Michele all' Adige, Italy
[f] Centre for Green Chemical Science, School of Chemical Sciences, University of Auckland, Private Bag 92019, Auckland, New Zealand

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ABSTRACT

Varietal thiols are important contributors to the aroma profile of a wide range of beverages including beer. A methodology for the simultaneous extraction and subsequent analysis of the varietal thiols 3-sulfanylhexan-1-ol (3SH), 3-sulfanylhexylacetate (3SHA) and 4-methyl-4-sulfanylpentan-2-one (4MSP), and the non-volatile precursors 3-*S*-cysteinylhexan-1-ol (Cys-3SH) and 3-*S*-glutathionylhexan-1-ol (GSH-3SH) in beer was developed and validated for the first time. The method, which utilises LC-MS/MS, was successfully tested on nine commercial beers, showing it to be widely applicable to this matrix. The syntheses of the novel internal standards d_8-3SH and d_8-3SHA, which allow a robust and accurate quantification of the hydronated congeners, are also reported. The simplicity of the QuEChERS-based (Quick, Easy, Cheap, Effective, Rugged and Safe) extraction allows for rapid, high throughput analyses suitable for both research and industry applications.

1. Introduction

Varietal thiols such as 3-sulfanylhexan-1-ol (3SH), 3-sulfanylhexylacetate (3SHA) and 4-methyl-4-sulfanylpentan-2-one (4MSP) are known to impart favourable tropical, fruity notes to food and drinks (Jeffery, 2016). These compounds have been detected in a range of fruits and beverages including grapefruit, passionfruit, guava, beer and wine (Cannon & Ho, 2018; Dennenlöhr, Thörner, & Rettberg, 2020; Engel & Tressl, 1991; Jeffery, 2016; Roland, Schneider, Razungles, & Cavelier, 2011). The very low sensory thresholds of these species mean that even a minute change in concentration can have significant sensory implications. While the biogenesis of these varietal thiols is still not fully understood, 3-*S*-cysteinylhexan-1-ol (Cys-3SH) and 3-*S*-glutathionyl hexan-1-ol (GSH-3SH) have been identified as precursors that can release 3SH during fermentation in winemaking.

Hops are known to be a source of thiol precursors and varietal thiols, the latter of which have been identified as key contributors to the overall characteristic aroma of New Zealand Nelson Sauvin hops, amongst others (Gros, Nizet, & Collin, 2011; Rettberg, Biendl, & Garbe, 2018). In contrast to the wine industry, where winemaking is somewhat restricted by the annual harvest of grapes, brewing has the advantage of comparatively short turn-around times and the possibility of all year-round brewing.

Vichi et al. proposed the simultaneous derivatisation and extraction of volatile thiols from alcoholic beverages using the selenium-based derivatising agent Ebselen (Vichi, Cortés-Francisco, & Caixach, 2015). Following this work, a recent publication by Tonidandel et al. described a method allowing the extraction and measurement of both non-volatile thiol precursors and their volatile free thiol counterparts in wine in a single chromatographic run (Tonidandel, Larcher, Barbero, Jelley, & Fedrizzi, 2021). The two families of compounds were previously routinely quantified using different analytical instrumentation owing to

Abbreviations: 1-Hexanethiol, (1-HT); 3-sulfanylhexan-1-ol, (3SH); 3-sulfanylhexylacetate, (3SHA); 3-*S*-cysteinylhexan-1-ol, (Cys-3SH); 3-*S*-glutathionylhexan-1-ol, (GSH-3SH); 4-methyl-4-sulfanylpentan-2-one, (4MSP); 2-phenyl-1,2-benzoselenazol-3-one, (Ebselen or Ebs).

* Corresponding author.
** Corresponding author. School of Chemical Sciences, University of Auckland, 23 Symonds St, Auckland, New Zealand.
E-mail addresses: rebecca.jelley@auckland.ac.nz (R.E. Jelley), b.fedrizzi@auckland.ac.nz (B. Fedrizzi).

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their difference in volatility. The use of a single instrument paves the way for high throughput analysis of these important families of compounds beyond the wine matrix. An increase in demand for 'fashionable' tropical and fruity aromas in beers has led to an increasing need by the brewing industry for rapid quantitation of these species and their precursors (Dennenlöhr et al., 2020). The use of a QuEChERS-based methodology (Quick, Easy, Cheap, Effective, Rugged and Safe) provides a simple and efficient platform for extraction (Anastassiades, Lehotay, Stajnbaher, & Schenck, 2003; Rejczak & Tuzimski, 2015). QuEChERS extractions exploit salting-out partitioning to attain desirable selectivity, sensitivity and specificity for the isolation of low concentration analytes. Herein we report what we believe to be the first optimisation of a QuEChERS-based extraction procedure that allows for concurrent LC-MS/MS analysis of free thiols and thiol precursors in beer.

2. Materials and methods

2.1. Chemicals and reagents

Acetonitrile (HPLC grade) and absolute ethanol (purity >99.8%) were purchased from ECP Laboratory and Research Chemicals (Auckland, New Zealand). Formic acid (LC/MS grade) was purchased from Thermo Fisher Scientific New Zealand (Auckland, New Zealand). Anhydrous magnesium sulfate (purity >99.5%), sodium chloride (purity >99.5%), sodium citrate dibasic sesquihydrate (purity >99%), and sodium citrate tribasic dehydrate (>99%) were purchased from Sigma-Aldrich New Zealand (Auckland, New Zealand). Type 1 water was obtained from a Barnstead NANOpure®DIamond™ system (Thermo Scientific, Waltham, USA).

Ebselen (2-phenyl-1,2-benzisoselenazol-3(2H)-one (98% purity) was purchased from Thermo Fisher Scientific New Zealand (Auckland, New Zealand). 3-sulfanylhexan-1-ol (3SH), 3-sulfanylhexyl acetate (3SHA) and 4-methyl-4-sulfanylpentan-2-one (4MSP) were obtained from Oxford Chemicals (Hartlepool, United Kingdom) and all standards had a minimum purity of 98%. 1-Hexanethiol was purchased from Sigma-Aldrich New Zealand (Auckland, New Zealand). d_3-(*R/S*)-3-*S*-cysteinylhexan-1-ol (d_3-Cys-3SH), and d_3-(*R/S*)-glutathionylhexan-1-ol (d_3-GSH-3SH) were supplied by Buchem B.V. (Apeldoorn, The Netherlands).

2.2. Internal standard syntheses

Cys-3SH and GSH-3SH were synthesised according to the method of Jelley et al. (Jelley, Duhamel, Barker, & Fedrizzi, 2020). The new compounds d_8-3SH and d_8-3SHA were synthesised using methods adapted from the literature; full experimental details, characterisation data and NMR spectra can be found in the Supplementary Information (Hebditch, Nicolau, & Brimble, 2007; Jelley, Duhamel, et al., 2020; Muhl, Pilkington, & Deed, 2020).

2.3. Ebs-adduct syntheses and characterisation

3SH, 3SHA, 4MSP, d_8-3SH, d_8-3SHA and 1-HT were reacted with Ebs (Ebselen, 5 mg thiol, 1 eq. Ebs) in 1 mL DCM, and the solutions were vortexed for 1 min before being concentrated *in vacuo*. ^{13}C and ^{1}H NMR spectra and HRMS data of the crude reaction mixtures were obtained and characterisation details for these Ebs adducts can be found in the Supplementary Information. While the mass spectra of a number of these adducts have been previously detailed, this is the first report of their structural characterisation.

2.4. Extraction and derivatisation procedure

Three extraction solvents (EtOH, ACN, and EtOH/ACN (1:1)), three extraction solvent volumes (6 mL, 8 mL and 10 mL), two modes of agitation (stirring using a magnetic stirrer bar and vortexing) and two Ebs derivatisation orders (derivatisation of free thiols before or after QuEChERS extraction) were investigated using a full factorial experimental design. The optimal conditions are described in the following experimental procedure.

Beer was degassed by gravity filtration using a folded filter paper-lined funnel. An aliquot of internal standard mix was added to degassed beer (25 mL) to give d_8-3SH (1000 ng/L); d_8-3SHA (500 ng/L); 1-HT (3000 ng/L); d_3-Cys-3SH (20 μg/L); d_3-GSH-3SH (20 μg/L) and the mixture was stirred at 200 rpm at room temperature for 2 min. ACN/EtOH (1:1 (mL/mL), 6 mL) laced with Ebs (0.6 mg/mL), was added and the solution was stirred for 2 min. A pre-weighed QuEChERS salt mix (magnesium sulfate (12 g), sodium chloride (3 g), sodium citrate tribasic dehydrate (3 g), sodium citrate dibasic sesquihydrate (1.5 g)) was added to the stirred solution and the mixture was stirred for an additional 10 min. The mixture was centrifuged at 1132×*g* at room temperature. An aliquot of the well-defined organic layer was filtered (PVDF; 0.22 μm) prior to LC-MS/MS injection.

2.5. UHPLC-MS/MS conditions

Thiol precursors and thiol-Ebs adducts were identified and quantified using modifications to a previously reported LC-MS/MS method (Jelley et al., 2016) using an Agilent 6460 Triple Quadrupole LC/MS system with an Agilent 1290 Infinity LC (California, U.S.A). An AJS ESI ion source was used, with a gas temperature of 300 °C, sheath gas

Table 1
MRM transitions for each compound analysed. Quantifier transition is denoted in bold. FV, Fragmentation Voltage; CV, Cell Accelerator Voltage; CE, Collision Energy; DT, Dwell Time.

Compound	RT (min)	MRM (*m/z*)	FV (V)	CV (V)	CE (eV)	DT (ms)
3SH-Ebs	14.08	**410 > 276**	135	7	15	50
		410 > 196	135	7	35	50
		408 > 274	135	7	15	50
d_8-3SH-ebs	14.07	**418 > 276**	135	7	15	50
		418 > 196	135	7	35	50
		406 > 274	135	7	15	50
3SHA-ebs	14.80	**452 > 276**	135	7	15	50
		452 > 196	135	7	35	50
		450 > 274	135	7	15	50
d_8-3SHA-ebs	14.79	**460 > 276**	135	7	15	50
		460 > 196	135	7	35	50
		458 > 274	135	7	15	50
4MSP-ebs	14.26	**408 > 276**	135	7	15	50
		408 > 196	135	7	35	50
		406 > 274	135	7	15	50
1-HT-ebs	15.77	**394 > 276**	135	7	15	50
		394 > 196	135	7	25	50
		392 > 274	135	7	15	50
Cys-3SH	9.11, 9.27	**222 > 205**	120	5	5	80
		222 > 101	120	5	16	80
		222 > 83	120	5	40	80
d_3-Cys-3SH	9.05, 9.21	**225 > 208**	120	5	5	80
		225 > 104	120	5	16	80
		225 > 86	120	5	40	80
GSH-3SH	11.21, 11.42	408 > 333	110	5	15	70
		408 > 279	110	5	8	70
		408 > 262	110	5	15	70
		408 > 162	110	5	22	70
d_3-GSH-3SH	11.18, 11.38	411 > 336	110	5	15	70
		411 > 282	110	5	8	70
		411 > 265	110	5	15	70

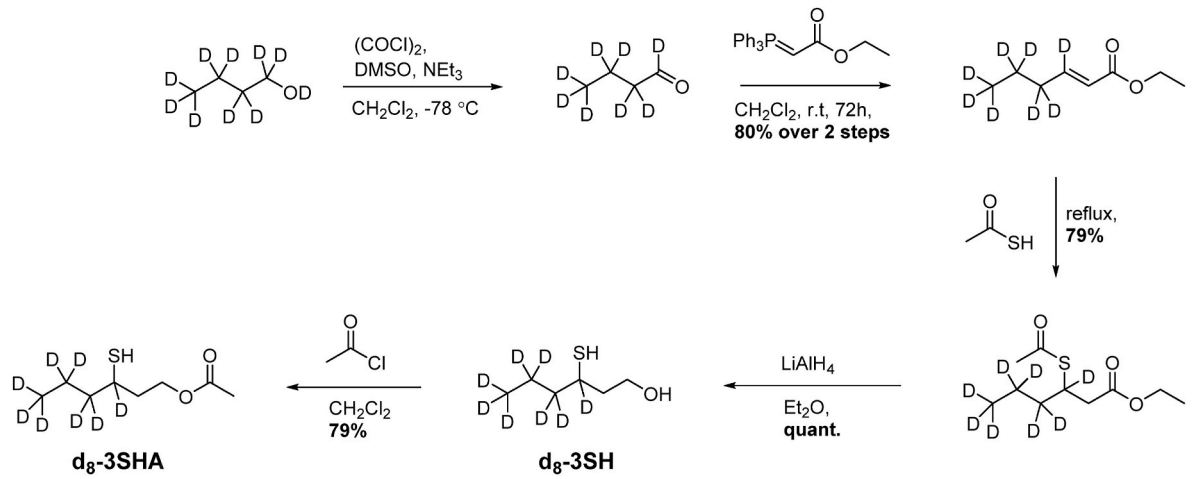

Fig. 1. Syntheses of the d_8-3SH and d_8-3SHA internal standards. Full experimental details can be found in the Supplementary Information.

temperature of 250 °C, a gas flow of 5 L/min, and a sheath gas flow of 11 L/min. A C18 Phenomenex Kinetex column (100 mm × 3 mm, 100 Å, 2.6 μm) (Phenomenex NZ Ltd, Auckland, New Zealand) operating at 25 °C with a sample injection volume of 5 μL was used. The solvent system was 0.1% formic acid in Milli-Q water (solvent A) and 100% acetonitrile (solvent B) with a flow rate of 0.5 mL/min. The gradient for solvent B over the 20 min run was as follows: 0 min 0%, 6 min 5%, 10 min 15%, 12 min 80%, 15 min 100%, and 20 min 5%. Detection was carried out in Multiple Reaction Monitoring (MRM) mode using the transitions outlined in Table 1.

2.6. Calibration and limits of detection

d_3-Cys-3SH and d_3-GSH-3SH were used as internal standards for the quantification of Cys-3SH and GSH-3SH, respectively. d_8-3SH and d_8-3SHA were used to quantify 3SH and 3SHA, respectively, and 1-HT was used as an internal standard for 4MSP. Calibration curves were prepared by spiking a beer sample (lager; pH, 4.01; alc, 4.0%) and were undertaken in triplicate, giving R^2 values greater than 0.981. The limit of detection (LOD) for each analyte of interest was calculated based on the standard deviation of the response; LOD = 3.3(Sd/S), where Sd = standard deviation of the response of the curve and S = the slope of the calibration curve (Allegrini & Olivieri, 2014).

2.7. Matrix effect – apparent recovery

Three beer matrices, a lager (4.0% alc), a pilsner (4.9% alc) and an India pale ale (IPA, 6.5% alc), were spiked with low concentrations (3SH (1000 ng/L), 3SHA (200 ng/L), 4MSP (50 ng/L), Cys-3SH (20 μg/L), GSH-3SH (28 μg/L)) and high concentrations (3SH (3000 ng/L), 3SHA (1000 ng/L), 4MSP (500 ng/L), Cys-3SH (40 μg/L), GSH-3SH (56 μg/L)) of the five target compounds. The six spiked matrices and three blank control matrices were subjected to the optimised extraction method (Section 2.3) in triplicate prior to LC-MS/MS analysis.

3. Results and discussion

3.1. d_8-3SH and d_8-3SHA syntheses

d_2-3SH and d_2-3SHA are commonly employed as internal standards for the quantification of 3SH and 3SHA in a variety of matrices using mass spectrometry-based techniques (Herbst-Johnstone, Piano, Duhamel, Barker, & Fedrizzi, 2013; Jelley, Deed, et al., 2020; Parish, Herbst-Johnstone, Bouda, Klaere, & Fedrizzi, 2016; Parish-Virtue, Herbst-Johnstone, Bouda, & Fedrizzi, 2019). Given the isotopic pattern of selenium (*i.e.* ^{78}Se and ^{80}Se), internal standards with a degree of deuteration greater than two are needed to quantify unlabelled Ebs-adducts of the target thiols. To address this, d_8-3SH and d_8-3SHA were synthesised in four and five steps, respectively, from commercially available d_{10}-butan-1-ol (Fig. 1). The methods employed were adapted from the syntheses of d_2-3SH and d_2-3SHA reported by Hebditch et al. (Hebditch et al., 2007) and the syntheses of d_8-Cys-3SH and d_8-GSH-3SH reported by Jelley et al. (Jelley, Duhamel, et al., 2020). All compounds were characterised using ^{1}H and ^{13}C NMR spectroscopy, IR spectroscopy, and HRMS, and the data were compared to literature where available. Full experimental procedures and characterisation of these new compounds can be found in the Supplementary Information.

3.2. Thiol-Ebs adducts

All six synthesised Ebs-adducts, and four thiol precursors (Cys-3SH, d_3-Cys-3SH, d_3-GSH-3SH and GSH-3SH), were measured in Scan mode to identify retention times, and then in Product Ion (PI) mode to identify optimal mass spectral parameters in order to quantify the species in MRM mode. Detailed mass spectral parameters can be found in Table 1.

3.3. Method optimisation

Acetonitrile is extensively reported in the literature as the 'go-to' extracting solvent for the QuEChERS method since it was first reported by Anastassiades et al. (Anastassiades et al., 2003) for the determination of pesticides in produce (Rejczak & Tuzimski, 2015). Recently, Tonidandel et al. investigated the suitability of ACN, EtOH and EtOAc for the extraction of thiol precursors and Ebs adducts from wine matrices (Tonidandel et al., 2021). ACN was confirmed as the most suitable extracting solvent for this matrix; however, EtOH was a close second. For this reason, and given beer contains a lower EtOH content than wine, acetonitrile, ethanol and a 1:1 (mL/mL) mixture of these two solvents were selected as candidates for further investigation. As shown in Fig. 2, it is clear that ACN is not the most suitable solvent for the extraction of thiol precursors from this matrix. However, in general, this solvent tends to extract the Ebs-derived thiols relatively well. EtOH behaves in the opposite manner displaying good extraction of thiol precursors but fairly poor extraction of the Ebs-derivatised thiols. The 1:1 mixture of EtOH and ACN provides a desirable compromise of the two extracting solvents and can be seen to adequately extract both families of compounds from the matrix. Combinations 8 and 26 using this 1:1 mix (Fig. 2) in particular performed comparably to or better than ACN counterparts for the extraction of the varietal thiols.

In most cases, stirring using a magnetic stir bar (10 min) gave better results than vortexing (30 s) in terms of the mode of agitation. While this could simply be a result of the extraction time difference, one issue that

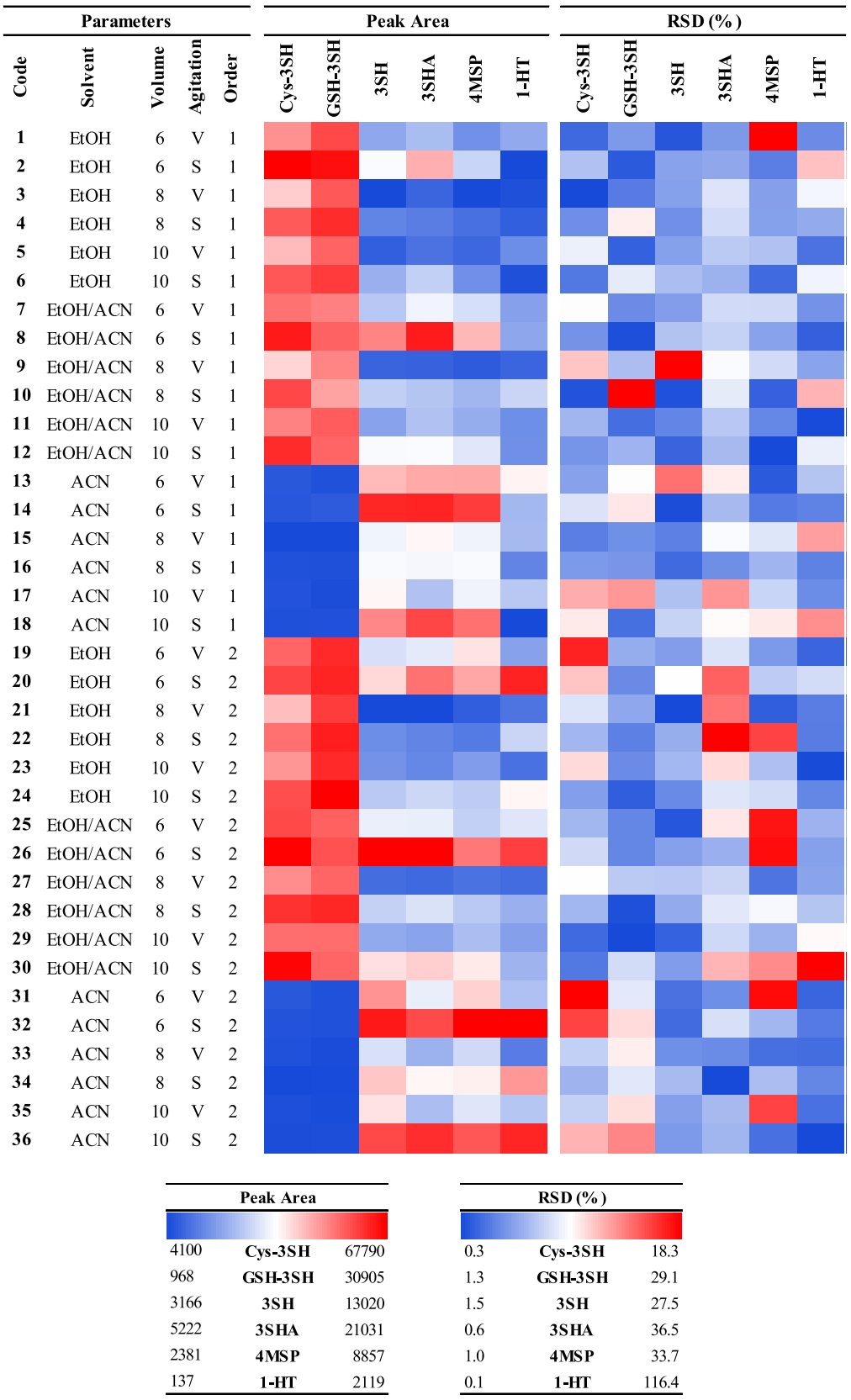

Fig. 2. Optimisation of QuEChERS extraction for beer (36 combinations in triplicate). Extracting solvent (EtOH, ACN, or EtOH:ACN 1:1), solvent volume (6, 8, or 10 mL), agitation (stirring (s), or vortex (v)), Ebselen derivatisation order (1 post-extraction, or 2 pre-extraction). LC-MS/MS peak area and % RSD are reported. Blue depicts low values, red depicts high values. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

became apparent with the vortexing approach was the formation of an emulsion-type mixture that often did not separate into two well-defined phases after centrifugation. The time saved on sample agitation, using a vortex for just 30 s, was often lost trying to carefully isolate the organic layer which was often far smaller in volume. In addition to this, the use of a multi-position stirrer plate meant that multiple samples could be stirring while focus turned to preparing the next set of batches. In terms of developing a protocol suitable for high throughput analysis using standard laboratory equipment, the stirring method, despite taking more time on a per sample basis, was more favourable from a practical perspective.

Given the extracting solvents selected for investigation were acetonitrile, ethanol and a 1:1 combination thereof, the organic layer could be injected directly into the LC-MS/MS. This avoided the need for a time-consuming concentration step and then re-dissolving the material in an injection-suitable solvent. An extracting solvent volume of 6 mL was found to give the highest concentrations of both thiol precursors and Ebs derivatised thiols. Given that the samples are injected directly from a sample of the organic layer, this result is expected. Volumes lower than 6 mL were investigated in preliminary trials but were found to give no or very inconsistent separation of the two layers. As a result, 6 mL was deemed to be the minimum working volume under these conditions and was shown in this optimisation study to give the highest concentration of the target compounds.

The derivatisation of free thiols recovered from a matrix using previously reported QuEChERS-based methods tends to be carried out after the extraction and isolation of the organic layer containing them (Román, Tonidandel, Larcher, Celotti, & Nicolini, 2018). It was thought that by spiking the extracting solvent with the derivatising agent (Ebs), the volatile thiols would be derivatised earlier on in the protocol, minimising any unwanted loss of analyte. This would also remove one step from the commonly employed general procedure, again making this method more suitable for high throughput analysis. Derivatisation order 1 (DO1) involved the organic layer being isolated post-centrifugation and reacted with Ebs over a 1 min period prior to syringe filtration and LC-MS/MS injection. Derivatisation Order 2 (DO2) derivatised free thiols by lacing the extracting solvent with Ebs. Fig. 2 reveals that there are few obvious differences between the two approaches, DO2 works just as well as its more laborious DO1 counterpart.

The beer sample volume of 25 mL was chosen because it could be measured efficiently using readily available laboratory glassware (25 mL volumetric flasks). This is a smaller sample volume than usually employed for thiol analysis in wine (for example, 50 mL for Herbst-Johnstone et al.'s (Herbst-Johnstone et al., 2013) ethyl propiolate derivatisation GC-MS method; 35 mL for Tonidandel et al. (Tonidandel et al., 2021) and 50 mL for Herbst-Johnstone et al.'s (Herbst-Johnstone, Nicolau, & Kilmartin, 2011) 4-hydroxymercuribenzoic acid (p-HMB) derivatisation GC-MS method). However, given that sufficient sensitivity could be achieved at this sample volume in preliminary trials and the use of small volumes is beneficial to the sample provider, 25 mL was deemed appropriate for the optimisation study.

The data obtained for this full factorial design were also analysed using the Design of Experiment function of OriginLab 2021 (OriginLab Corporation, Northampton, MA, USA), calculating the desirability function of the different combinations tested; the optimisation of the responses also yielded combination C26 (EtOH/ACN, 6 mL, stirring, DO2) as the best experimental conditions for the analysis of the five target molecules.

Combination C26, (EtOH:ACN (1:1); 6 mL; stirring; DO2) was selected as the best set of conditions for the extraction of varietal thiols and their precursors from beer.

3.4. Method validation

Calibration curves were prepared by analysing beer samples spiked with the five target compounds. These spiked matrices were extracted according to the aforementioned optimised method (C26, Section 3.3). Triplicate analyses were performed for each level. Linearity was evaluated and all compounds gave a calibration curve with an R^2 value greater than 0.981 (Table 2). Limit of detections (LOD) for the five compounds of interest were suitable for the quantification of these molecules in real beer. LODs for Cys-3SH and GSH-3SH were lower than those reported by Tonidandel et al. in a wine matrix but slightly higher for the three varietal thiols (Tonidandel et al., 2021). In order to assess the repeatability of the method, a real beer matrix was spiked with low, medium and high concentrations of the five target species. The samples were prepared in triplicate and their RSD% are presented in Table 2. Values ranged from 0.61 to 10% for varietal thiols and from 1.1 to 8.8% for thiol precursors.

To assess the effect of the matrix on the extraction of the five target species, the apparent recovery of these compounds from three different beer styles was investigated. The matrix was spiked at 'high' and 'low' concentrations appropriate to the particular compound and this was compared to the measurements obtained for the unadulterated matrix (Table 3). Good apparent recoveries were obtained for the levels tested as well as for all three beer matrices considered in this study.

Table 3
Apparent recovery (%) for the five compounds of interest in three different beer styles. N replicates = 3.

Matrix	Level[a]	3SH	3SHA	4MSP	GSH-3SH	Cys-3SH
Pilsner	Low	84.7	90.8	89.6	109.3	117.8
	High	89.3	92.7	88.6	95.5	105.4
IPA	Low	91.7	94.0	77.5	97.9	116.4
	High	90.2	105.9	66.6	122.9	118.7
Lager	Low	103.9	94.4	75.1	96.1	116.5
	High	119.4	110.2	95.4	110.5	114.7

[a] Concentration levels in spiked beer samples: Low (3SH (1000 ng/L), 3SHA (200 ng/L), 4MSP (50 ng/L), Cys-3SH (20 μg/L), GSH-3SH (28 μg/L)); High (3SH (3000 ng/L), 3SHA (1000 ng/L), 4MSP (500 ng/L), Cys-3SH (40 μg/L), GSH-3SH (56 μg/L)).

Table 2
O.T. (odour threshold), Repeatability (RSD %), limits of detection (thiols, ng/L; precursors, μg/L), and linearity of the method. N = number of replicated samples.

Compound	O.T. in beer (ng/L)	I.S.	Repeatability (N = 3, RSD %)			Calibration Curves		
			Low	Med	High	Linear range (μg/L)	R^2	LOD (μg/L)
3SH	55 (Tran, Cibaka, & Collin, 2015)	d_8-3SH	0.61	3.2	7.3	0.025–2	0.9862	0.030
3SHA	5 (Kishimoto, Morimoto, Kobayashi, Yako, & Wanikawa, 2008)	d_8-3SHA	1.5	7.1	8.5	0.01–1	0.9977	0.0069
4MSP	1.5 (Tran et al., 2015)	1-HT	11	4.9	10	0.01–1	0.9815	0.025
GSH-3SH	–	d_3-GSH-3SH	1.1	3.8	8.8	0.5–84	0.9971	0.61
Cys-3SH	–	d_3-Cys-3SH	2.5	3.0	5.6	0.5–50	0.9979	0.48

Low: 3SH (200 ng/L), 3SHA (50 ng/L), 4MSP (50 ng/L), Cys-3SH (5 μg/L), GSH-3SH (7 μg/L).
Med: 3SH (1000 ng/L), 3SHA (200 ng/L), 4MSP (200 ng/L), Cys-3SH (20 μg/L), GSH-3SH (28 μg/L).
High: 3SH (3000 ng/L), 3SHA (1000 ng/L), 4MSP (500 ng/L), Cys-3SH (40 μg/L), GSH-3SH (56 μg/L).

Table 4
Method application to nine commercial beers. Concentrations are reported in ng/L as an average of three replicates ± Standard deviation (s.d.).

Style	Alc %	pH	3SH	±s.d.	3SHA	±s.d.	4MMP	±s.d.	GSH-3SH	±s.d.	Cys-3SH	±s.d.
IPA	6.20	4.6	3515	201	21.4	0.4	253.1	14.1	35533	1439	23823	845
IPA	6.50	4.8	345	26	4.9	1.5	36.1	1.3	34976	983	32961	1254
IPA	5.80	4.0	1521	90	8.0	2.0	103.5	20.6	38346	2412	22703	1261
Lager	4.00	4.0	2213	67	24.6	2.0	13.5	1.8	24639	953	23284	1022
Lager	4.50	4.3	930	242	22.1	2.3	3.3	1.2	23062	1718	23800	1691
Pale Ale	4.60	4.2	2971	201	20.1	1.5	180.4	42.1	34596	2942	13523	384
Pale Ale	4.60	4.4	598	7	20.0	2.4	25.4	4.2	54694	3493	19704	1092
Pilsner	4.90	4.3	426	27	12.8	0.2	14.8	2.7	44574	2890	21658	787
Wheat Beer	4.90	4.4	1770	42	4.2	0.2	9.4	2.2	338	24	2734	102

3.5. Method application

To verify the applicability of the aforementioned validated method, nine commercial beers, of varying style, were analysed in triplicate for their varietal thiol and thiol precursor content. Table 4 shows the results of this quantitation. All five target compounds were detected and measured in each of the nine commercial samples. The wheat beer sample contained the lowest concentrations of the thiol precursors Cys-3SH and GSH-3SH of the sample analysed. The concentration of GSH-3SH in this sample was more than 162-fold lower than the average concentration measured in the Pilsner sample having the highest concentration of the sample set (54.7 μg/L). Collin et al. (Gros et al., 2011) previously reported the concentrations of 4MSP and 3SH in four ale beers hopped with different cultivars (Tomahawk, Nelson Sauvin, Cascade and Saaz). 4MSP was reported at similar concentrations to the samples measured in this study. On the other hand, the concentration of 3SH was lower (ranging from 32 to 243 ng/L) in the experimental beers reported by Collin et al. compared to the nine commercial beers included in this study (Table 4).

4. Conclusion

This study has reported the first optimisation of a convenient and versatile QuEChERS-based extraction protocol capable of measuring both varietal thiols (3SH, 3SHA, 4MSP) and their non-volatile precursors (Cys-3SH, GSH-3SH) in beer using LC-MS/MS. In addition, the syntheses and characterisations of two new internal standards, d_8-3SH and d_8-3SHA, have been reported. The analytical method exhibited good linearity and apparent recoveries. Repeatability (% RSD) ranged from 0.61 to 10% for all five target analytes and apparent recovery from 67 to 123%. The method was then successfully applied to nine commercial beer samples showing it is fit for purpose and importantly provides a sound platform for the identification and analyses of other beer related thiols and precursors should standards be available.

CRediT authorship contribution statement

Rebecca E. Jelley: Methodology, Investigation, Formal analysis, Supervision, Writing – original draft. **Hayden Jones-Moore:** Investigation. **Angela Guan:** Investigation. **Chloe Z.-J. Ren:** Investigation. **Jack L.-Y. Chen:** Methodology, Supervision. **Loris Tonidandel:** Writing – review & editing. **Roberto Larcher:** Writing – review & editing. **Bruno Fedrizzi:** Supervision, Formal analysis, Writing – review & editing.

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2022.113563.

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