Journal of Chromatography A, 1218 (2011) 875–881

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Comparison of volatile constituents extracted from model grape juice and model wine by stir bar sorptive extraction–gas chromatography–mass spectrometry

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ARTICLE INFO

Article history:
Received 18 October 2010
Received in revised form
15 December 2010
Accepted 16 December 2010
Available online 23 December 2010

Keywords:
Stir bar sorptive extraction
Thermal desorption
Volatile organic compounds
Model wine

ABSTRACT

A stir bar sorptive extraction (SBSE) method coupled with gas chromatography–mass spectrometry was optimised for the analysis of volatile components of a model wine, based on a previously optimised method used for analysis of the same components in model grape juice. The presence of ethanol in the model wine sample matrix resulted in decreased sensitivity of the method toward most of the volatile constituents. Mean percent relative recoveries and reproducibilities (%CV) were 22.8% and 7.1%, respectively, compared with 28.4% and 8.5% for model grape juice. The mean limit of detection (LoD) ratio (juice:wine) was 0.25. Similar sensitivities for the two sample matrices using this method were achieved by changing the split ratio from 20:1 (grape juice) to 5:1 (wine), giving a mean limit of detection ratio (juice:wine) of 1.0, thus allowing direct comparison of chromatograms of volatile components in the two matrices. This enabled direct comparisons of grape juices and the wines derived from them by alcoholic yeast fermentation. The influence of ethanol concentration in the range 9–15% on method sensitivity is discussed, using an overlay of the total ion chromatograms. The use of a gas saver device for the 5:1 split ratio analysis of desorbed model wine aroma compounds is discussed in terms of preventing extraneous reaction of sorbent and stationary phases with air during analysis.

1. Introduction

Stir bar sorptive extraction (SBSE) [1,2] is a sensitive technique for the separation and focusing of organic components from an aqueous or partially aqueous matrix prior to their analysis, usually by gas chromatography/mass spectrometry. The method uses a silica stir bar (the Twister™) onto which the sorbent phase, usually polydimethylsiloxane (PDMS), has been bonded. The stir bar is stirred for a certain time in the sample. After sampling, the stir bar is placed in a thermal desorption unit, coupled on-line to a gas chromatograph. Like solid phase microextraction (SPME), SBSE is a non-destructive, reliable, robust and generally speedy method that has reasonable reproducibility and analyte recovery when applied to the extraction of volatile and other components from aqueous solution. It has been shown to be more sensitive than SPME in the analysis of various wine components [3], because the volume of sorbent phase (typically 24 μl on a 10 mm stir bar) is much greater than that found on SPME fibres (typically 0.5 μl). Likewise, SBSE has been shown to be more sensitive than a classical microscale simultaneous distillation–extraction (SDE) method when applied to the volatile components of grape juice [4]. SBSE has been used in the analysis of volatile components (aroma compounds) of alcoholic beverages [3,5–7,9–13].

Aroma is an important property of most alcoholic beverages and is the result of the contribution of a large number of volatile and semi-volatile constituents, many of which are present in concentrations lower than $\mu g\,l^{-1}$ [14]. With respect to wine aroma compounds, some are derived purely from the original unfermented grape juice, many others are derived solely from the wine making process (fermentation, maturation in oak vessels, etc.) and yet others have a duel origin: they are present in the unfermented grape juice and are also end-products of yeast metabolism during alcoholic fermentation [15]. As a result of the alcoholic fermentation of grape juice, ethanol becomes the single most abundant organic compound in wine, thus presenting a very different matrix of aroma compounds to the extraction/focusing method. There is scant literature on the influence of ethanol on sorbent extraction sensitivity toward aroma compounds, although it has been demonstrated that in the higher ethanol range of rums and vodkas, SPME sensitivity decreases with increase in ethanol content [16].

This paper presents a comparison of the ability of PDMS stir bars to extract volatile compounds from grape juice and wine matrices. The major aim of this work was to use a previ-

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doi:10.1016/j.chroma.2010.12.078

ously optimised SBSE method and develop the subsequent gas chromatographic–mass spectrometric analytical method for the determination of volatile components in a model wine containing 12% ethanol (v/v). Thus, semi-quantitative data can be compared directly with those obtained for the same volatile components in an identical model grape juice (the model wine without the ethanol). As a result of this, the optimised method can then be applied to the direct comparison of the aroma profile of a real wine and the grape juice from which it was derived. This work also reports briefly on the influence of ethanol content in the range 9–15% (v/v) on the effectiveness of extraction of volatile compounds on PDMS stir bars. Finally, there is a discussion on the use of the Agilent "gas saver" for the 5:1 split ratio analysis of desorbed model wine aroma compounds in light of the prevention of extraneous exposure of sorption or stationary phases to air.

2. Experimental

2.1. Materials

Acetone and water ("super pure" quality) were acquired from Romil (Cambridge, UK). *n*-Tetradecane 99+% was obtained from Aldrich (Gillingham, UK). Standard Twister™ stir bars coated with 24 μl of PDMS (length: 10 mm, film thickness: 0.5 mm) were obtained from Gerstel (Mülheim an der Ruhr, Germany). All aroma compounds were in-house (Frutarom (UK) Ltd.) samples. Ethanol (99.7–100% v/v) was AnalaR™ grade (BDH, Poole, UK). All other reagents were of analytical food quality.

Model wines were created according to the literature [17]. Tartaric acid (0.70 g), potassium bitartrate (1.15 g) and ethanol (90–150 ml, for final ethanol concentrations of 9–15% v/v) were dissolved in "super pure" water and made up to 1 l with this water in a volumetric flask.

For the production of the synthetic grape aroma mix, equal masses (0.1 g) of 46 typical grape aroma compounds [4,18] were added together and mixed. Synthetic wine to be used for optimisation and method validation was made by mixing a small volume of diluted aroma mix with model wine, as described in Section 2.2.

2.2. Sampling conditions for SBSE

For SBSE analysis, each model wine (20 ml) was spiked with synthetic aroma mix (1% w/v in acetone, 1 μl) in a 20 ml headspace vial. Therefore, the concentration of each aroma component was *ca.* 10 μg/l (i.e. identical concentrations to those used in the SBSE extraction of grape juice validation [4]). Prior to use, the stir bars were conditioned at 300 °C in a helium stream (100 ml/min) for 1 h using a TC-1 tube conditioner (Gerstel). A pre-conditioned Twister™ stir bar was added to each of the sample vials before being capped and placed onto a Gerstel Twister™ stirrer plate (TS-1). Samples were stirred under the previously optimised ambient temperature juice conditions (1000 rpm for 2 h [4]). On completion, stir bars were removed from the vials, washed with "super pure" water (5 ml) and blotted dry on a lint free tissue. The stir bars were finally spiked directly with internal standard solution (*n*-tetradecane 0.02% w/v in acetone, 1 μl) then transferred to a clean pre-conditioned thermal desorption tube and placed onto a TDS-A autosampler for analysis. Addition of *n*-tetradecane directly to the stir-bar allowed it to be used for semi-quantifying the extracted aroma compounds, i.e. it acts as both an extraction internal standard and a GC internal standard, since its recovery into the stir-bar PDMS phase is 100% [4]. All samples were analysed (in triplicate) using the optimised conditions as previously described [4].

2.3. Instrumentation and conditions

For an account of the method development and optimisation of the TD – GC/MS procedure, see [4]. The SBSE analyses were performed using an automated TDS-2/TDS-A thermal desorption unit (Gerstel) mounted on an Agilent 6890 gas chromatograph system coupled to a quadrupole Agilent 5973 electron ionisation (70 eV) mass spectrometric detector (Agilent Technologies, Palo Alto, CA, USA) equipped with an Agilent Innowax (crossed linked polyethylene glycol) capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness). The carrier gas was helium with a constant column flow rate of 1 ml/min (mean velocity 36 cm/s).

The analytes were cryofocused in a programmed temperature vaporising injector (PTV) (CIS-4, Gerstel) held at −50 °C with liquid nitrogen prior to injection. A packed liner containing 20 mg of Tenax TA was used in the PTV. Stir bars were thermally desorbed in a stream of helium carrier gas at a flow rate of 70 ml/min and by programming the TDS 2 from 20 °C to 300 °C (5 min) at a rate of 60 °C/min. After desorption and cryofocusing, the CIS-4 was programmed from −50 °C to 260 °C (20 min) at 12 °C/s to transfer the trapped aroma volatiles onto the analytical column. The TDS-2 was operated in the splitless mode, whereas the CIS-4 was operated in the split mode to provide either a 50:1 injection split ratio for recovery experiments or 20:1 and 5:1 split ratios for limits of detection experiments. The GC oven temperature was programmed from 40 °C (5 min) to 240 °C (20 min) by increasing the temperature at 3 °C/min and the MS was operated in selected ion monitoring mode (SIM) with a dwell time of 100 ms (for all ions) for recovery experiments, and in scan mode (35–300 amu) for limits of detection experiments. The temperature of the MSD transfer line was retained at 250 °C throughout.

The SBSE-GC/MS determinations were performed in triplicate. Relative recoveries (%RR) ranged from 0.05% (for acetoin) to 122% (for δ-3-carene). Coefficients of variance (CV) ranged from 2.5% (for hexyl butyrate) to 37.8% (for acetoin) (mean RSD = 4.6%).

3. Discussion

3.1. Comparison of SBSE extraction of aroma compounds from model juice and model wine

Recoveries were determined by comparison of the peak area response ratio of each aroma constituent relative to the internal standard from the extracts with the same response ratios obtained from its corresponding standard. The relative recoveries produced from the stir bar sorptive extraction technique [4] applied to the extraction of grape/wine aroma compounds from model wine are in the range of 0.05–125% (average recovery 22.8%). The coefficients of variation are in the range of 2.5–37.8% (average 7.1%). This compares with recovery range 0.3–110% (average recovery 28.4%) and coefficients of variation range 1.2–63.9% (average 8.5%) for extraction of the same aroma compounds from model grape juice, under identical conditions [4].

It can be seen from Table 1 that the stir bar sorption extraction efficiency (as measured by % relative recovery) of model wine compounds belonging to the same chemical series generally increases with increase in $\log K_{o/w}$ values. For example, a low recovery (0.3%) was obtained for isobutyric acid ($\log K_{o/w} = 1.00$), whereas the recovery for dodecanoic acid ($\log K_{o/w} = 5.00$) was 31%. The same feature was observed in the SBSE of model juice compounds [4]. With the introduction of ethanol to the sample matrix in the model wine, the relative recoveries for the aldehydes increased when compared directly to those obtained from the model juice, as illustrated by the following examples. The recovery of hexanal ($\log K_{o/w} = 1.80$) increased from 5% in model juice to 19.5% in

Table 1
Mean relative recoveries (%RR), standard deviations (SD) and coefficients of variation (%CV) for extraction of synthetic grape/wine aroma compounds from model wine (12% ethanol by volume) by stir bar sorptive extraction (SBSE). Corresponding data for model grape juice [4] under identical conditions, are given in brackets.

Compound	$\log K_{o/w}$[a]	SBSE calculated recovery (%)[b]	(n = 11) %RR	SD	%CV
Ethyl acetate	0.86	0.9	0.5 (1.0)	0.07	12.9 (13.1)
2-Butanol	0.77	0.7	0.5 (0.4)	0.04	8.1 (13.0)
Ethyl butyrate	1.85	7.8	2.7 (14)	0.29	10.6 (11.5)
Butyl acetate	1.85	7.8	2.9 (15)	0.18	6.2 (9.6)
Hexanal	1.80	7.0	19.5 (5.0)	2.81	14.4 (12.2)
Isoamyl acetate	2.26	17.9	9.4 (37)	0.52	5.5 (6.7)
δ-3-Carene	4.61	98.0	122 (100)	5.07	4.2 (7.7)
1-Butanol	0.84	0.8	0.84 (0.9)	0.035	4.2 (15.8)
β-myrcene	4.88	98.9	112 (100)	5.16	4.6 (7.2)
α-Terpinene	4.75	98.5	59 (46)	2.63	4.5 (13.2)
Amyl methyl ketone (2-heptanone)	1.73	6.1	3.6 (18)	0.12	3.3 (4.6)
Limonene	4.83	98.8	125 (110)	4.88	3.9 (6.3)
3-Methyl-1-butanol (isoamyl alcohol)	1.26	2.1	0.11 (0.50)	0.016	13.9 (9.6)
(*E*)-2-Hexenal	1.58	4.4	1.9 (2.9)	0.15	7.6 (3.9)
Hexyl acetate	2.83	44.8	34 (85)	0.97	2.8 (2.1)
3-Hydroxy-2-butanone (acetoin)	-0.36	0.1	0.05 (0.4)	0.02	37.8 (17.5)
Octanal	2.78	42.0	23 (2.9)	0.71	3.1(8.3)
(*E*)-2-Hexenyl acetate	2.61	32.8	18.8 (63)	0.48	2.6 (2.1)
1-Hexanol	1.82	7.3	2.5 (4)	0.14	5.6 (11.6)
(*Z*)-3-Hexen-1-ol	1.61	4.7	0.09 (0.7)	0.01	12.0 (9.7)
n-Tetradecane (internal standard)	7.22	100	100.00 (100.00)	0.00	0.0 (0.0)
Hexyl butyrate	3.81	88.6	80 (88)	2.00	2.5 (2.5)
3-(Methylthio) propanal (methional)	0.41	0.3	12.1(22)	0.64	5.2 (2.8)
Furfural	0.83	0.8	2.7 (3)	0.53	19.6 (39.4)
Octyl acetate	3.81	88.6	74 (80)	1.91	2.6 (3.0)
Decanal	3.76	87.4	46 (2.0)	1.59	3.5 (9.3)
Benzaldehyde	1.71	5.8	1.9 (0.8)	0.08	4.3 (13.5)
Linalool	3.38	74.2	2.8 (13.2)	0.09	3.1(2.2)
Isobutyric acid	1.00	1.2	0.3 (0.3)	0.06	22.5 (63.9)
β-Caryophyllene	6.30	100	63 (41)	2.47	3.9 (8.6)
Acetophenone	1.67	5.3	1.45 (5.4)	0.05	3.4 (2.7)
Neral	3.45	77.2	10 (17.6)	0.69	6.9 (1.2)
α-Terpineol	3.33	72.0	0.92 (5.2)	0.04	4.4 (2.9)
Neryl acetate/Geranial	4.48/3.45	97.3/77.2	29.7 (38)	0.92	3.1(1.5)
Valeric acid	1.56	4.2	0.13 (0.5)	0.01	7.1 (10.5)
Geranyl acetate	4.48	97.3	53 (66)	1.74	3.3 (1.6)
1-Decanol	3.79	88.1	20 (53)	0.64	3.2 (1.3)
β-Citronellol	3.56	81.3	4.1 (22.0)	0.19	4.7 (1.9)
Nerol	3.47	78.0	1.8 (12.6)	0.09	4.8 (2.3)
β-Damascenone	4.21	95.1	22 (49)	0.96	4.4 (1.3)
Geraniol	3.47	78.0	2.7 (10.9)	0.12	4.5 (2.0)
2-Phenylethanol	1.57	4.3	0.13 (0.40)	0.03	20.2 (7.8)
β-Ionone	4.29	95.9	29 (51)	0.90	3.1(2.9)
Nonanoic acid	3.52	79.9	1.8 (13)	0.08	4.5 (4.4)
Methyl anthranilate	2.26	17.9	1.1(2.7)	0.04	3.5 (3.7)
Dodecanoic acid (lauric acid)	5.00	99.2	31(69)	1.55	5.1(2.3)

[a] $\log K_{o/w}$ values as predicted from "SRC $K_{o/w}$Win" version 1.66.
[b] Calculated recoveries using "Gerstel Twister™ recovery calculator software" version 1.0.4.1 based on the use of a 10 mm × 0.5 mm stir bar (24 μL PDMS coating) and a 20 mL sample volume.

the model wine. This trend becomes more pronounced as $\log K_{o/w}$ increases, as shown by the recovery of octanal ($\log K_{o/w}$ = 2.78) being 2.9% in model juice, but 23% in model wine, and decanal ($\log K_{o/w}$ = 3.76): 2% (model juice), but 46% (model wine).

However, the presence of ethanol in the matrix (making it more organic, compared with the model juice matrix) results in lower relative recoveries for many other compounds. Thus the ester components, isoamyl acetate ($\log K_{o/w}$ = 2.26), hexyl acetate ($\log K_{o/w}$ = 2.83) and (*E*)-2-hexenyl acetate ($\log K_{o/w}$ = 2.61) show an average threefold decrease in relative recoveries when compared with the model juice matrix. Even greater decreases were observed for the alcohols, linalool ($\log K_{o/w}$ = 3.38), 1-decanol ($\log K_{o/w}$ = 3.79), β-citronellol ($\log K_{o/w}$ = 3.56), nerol ($\log K_{o/w}$ = 3.47), and geraniol ($\log K_{o/w}$ = 3.47). Here the average decrease in relative recovery was *ca.* fivefold. This can be explained by the fact that the presence of other organic solvents, such as ethanol, during extraction can be expected to reduce analyte partitioning between the solvent and the PDMS sorbent phase. The magnitude of the effect is related to the polarity and concentration of the solvent, and the polarity of the analytes of interest.

Matrix modification, such as addition of salts and alteration of medium pH, is a well-known technique for improving the effectiveness of liquid–liquid and sorbent extraction of volatile components from aqueous matrices [26]. However, a review of the literature for such methods employed in the extraction volatile compounds in wine and similar water/ethanol matrices revealed negative or inconclusive results. For example, the effect of salt addition was investigated for the determination of stale-flavour carbonyl compounds in beer by SBSE [8]. Here the authors noted that the responses obtained with 15% salt addition using a 10 ml sample was actually much lower for all analytes, being 0.16–0.67 times those obtained with no salt addition. Likewise, it has been shown that addition of salt did not increase the recoveries of volatile phenols in wines by SBSE [6]. Similar results were obtained in the extraction

and determination of volatile compounds in oak-aged wines [11] in the analysis of wine cork taint [22], in the analysis of trace amounts of off-flavour compounds in drinking water [23] and in the analysis of wine primary aroma compounds [12]. It is also interesting to note that sodium chloride (NaCl) additions added to samples in the application of SBSE for migration testing of food packaging materials, demonstrated that the extraction efficiency of the analytes studied (including octanal, limonene, nonanal and decanal) was reduced by addition of NaCl and ethanol [24]. These observations can be explained by the fact that water, which has a higher dielectric constant than ethanol, is more efficient in solvating the added salt. In effect, the analytes are "salted out" into an environment containing a higher percentage of ethanol. This results in higher solubility of the analytes in the salt-containing matrix, and consequently smaller amounts of analytes are extracted since adsorption increases with decreasing analyte solubility in the matrix, given the same coating [16].

Alteration of sample pH before SBSE analysis, as a way of matrix modification to improve extraction recoveries, has not been identified in the literature specifically for the analysis of volatile organic compounds in wine. It may be supposed that ionisable acidic organic species, such as phenols and carboxylic acids, are not readily partitioned into the non-polar PDMS phase of an SBSE "Twister™", and that partitioning could be improved by lowering the pH of the sample before extraction [25]. However, lowering the sample pH from 3.6 (normal wine pH) to pH 2 did not significantly enhance the extraction of chlorinated phenols into the stir bar phase [22]. The lack of literature examples of pH-lowering prior to SBSE analysis could be due to the fact that inorganic or mineral acids that are often used in analytical chemistry sample preparation for this purpose, can cause damage to gas chromatographic stationary phases, such as PDMS [19]. The PDMS coating of the stir bars may also be degraded over a period of time, like the coating of SPME fibres [26].

From the observations outlined above, sample matrix modification using the salting out and pH adjustment techniques were not investigated in this study. Instead, it was decided to improve the sensitivity by adjustment of the GC split ratio.

3.2. Comparison of LoD of aroma compounds from model juice and model wine and adjustment of GC split ratio for optimisation

The limit of detection (LoD) for each of the synthetic grape/wine aroma compounds was determined according to the LGC [27]. A fresh solution of the synthetic aroma mix (Section 2.1) was produced (0.10 g of each aroma compound) which was further diluted to a final volume of 10 ml with "super pure" acetone (to give a final concentration *ca.* 1% w/v of each aroma compound). Fresh model grape juice or wine (20 ml) was added to a clean, pre-conditioned 20 ml glass headspace vial and spiked with the above aroma mix solution (1 μl). Therefore the final concentration of each component in the model systems was *ca.* 0.5 mg/l. All samples were extracted as previously described (Section 2.2).

All samples were analysed ($n = 5$) *via* thermal desorption/GC-MSD using the optimised conditions as previously described [4] with the TDS-2 operated in the splitless mode and the CIS-4 operated in the split mode to provide either a 20:1 split ratio for model juice samples and 20:1 or 5:1 split ratios for model wine samples.

The above procedure was repeated using serial dilutions of the aroma mix (50:50 v/v dilutions with "super pure" acetone) until the limits of detection were determined for all 46 aroma components [27]. Blank analyses were performed in between each sample set by analysing empty clean pre-conditioned thermal desorption tubes (in duplicate). Model juice/wine blanks were also analysed (in duplicate) for each GC–MS sequence of analyses carried out.

As can be seen from Table 2, the presence of ethanol in the sample matrix in general, raised the limits of detection for a significant number of the aroma compounds. The average ratio (LoD model juice/LoD model wine) for the 46 aroma compounds under the 20:1 injection split ratio was shown to be 0.34, which indicated an average threefold increase in the overall method detection limits. Moreover, removal of all ratios of *ca.* 1.00 or above (i.e. those compounds that had the equivalent or lower LoD in model wine compared to model juice under 20:1 split injection) gave an overall average ratio of 0.25. This demonstrated that to achieve acceptable detection limits of typical aroma compounds extracted from the spiked 12% ethanol v/v containing matrix compared to grape juice, then the sensitivity of the "wine" SBSE method needed to be increased four fold.

Table 2 shows that a 5:1 split injection ratio reduced the LoDs of the vast majority of the most problematic aroma analytes in model wine in line with those obtained from SBSE of the spiked model grape juice with a 20:1 injection split ratio. For example, the ratio (LoD model juice/LoD model wine) for β-damascenone increased from 0.25 under the 20:1 split injection ratio to 1.00 under the 5:1 split conditions thus demonstrating that, for some analytes, an identical LoD could be obtained for analysis of juice and wine samples. Therefore this lower split ratio was adopted for all subsequent analysis of real wine samples for this study (to be discussed in detail in a future paper).

Unfortunately, the LoDs could not be reduced further for the most problematic aroma components of model wine by extraction of larger sample sizes. The total ion chromatograms (TIC) obtained from the triplicate SBSE analyses of 20/50 and 100 ml sample sizes (concentration of each synthetic aroma component *ca.* 10 μg/l) were compared and it was revealed that extraction of 50 and 100 ml samples over a typical 20 ml sample size offered no advantage for the compounds that needed the reduction in detection limit; only the most non-polar compounds with higher $\log K_{o/w}$ values (such as limonene, $\log K_{o/w} = 4.83$) benefited which was clearly evident on overlay comparison of the total ion chromatograms.

3.3. Effect of narrow variations in % ethanol (v/v) on SBSE recoveries

To evaluate the effects of ethanol concentration on extraction sensitivity, the total ion chromatograms (TIC) of extracts of the aroma compounds from model wines containing 9%, 10%, 11%, 12%, 13%, 14% and 15% ethanol (v/v) were compared using Agilent MS Chemstation data analysis software (overlaid). Out of the 46 aroma compounds of the synthetic grape/wine aroma mix, 8 components (hexyl acetate, octanal, (*E*)-2-hexenyl acetate, neryl acetate, geranyl acetate, 1-decanol, β-damascenone and β-ionone) showed noticeably lower peak area responses after SBSE from the model wine with 15% v/v ethanol, compared with the 9% alcohol model wine. For example geranyl acetate showed a 14% decrease in peak area response and 1-decanol showed a 32% decrease in peak area response, when compared to SBSE from the 9% ethanol model wine (Fig. 1). However, the differences observed on overlay comparison of the data obtained by SBSE of the model wines with ethanol concentrations equivalent to those of the real wine samples of this study (range 10–12% v/v, a detailed discussion is intended for a future paper) showed very little or no differences in peak area responses for the 46 aroma compounds; for example geranyl acetate showed only a 3% decrease in peak area response whereas 1-decanol, showed a 4% decrease in peak area response extracted from the 12% v/v model wine when compared directly to the 10% v/v model wine.

Comparison of the total ion chromatograms obtained by SBSE of a lower volume of the spiked 12% ethanol v/v model wine (10 ml of sample, instead of 20 ml – to lower the phase ratio between the

Table 2
Limit of detection (LoD) results for synthetic grape/wine aroma compounds extracted from model wine (12% ethanol by volume) compared to model grape juice using SBSE.

Injection split ratio Compound	log $K_{o/w}$	20:1 Split Model juice (MJ) LoD (μg/L)	20:1 Split Model wine (MW) LoD (μg/L)	5:1 Split Model wine (MW) LoD (μg/L)	20:1 Split Ratio (LoD MJ/LoD MW)	5:1 Split Ratio (LoD MJ/LoD MW)	Odour threshold (μg/L)[a]
Ethyl acetate	0.86	34	34	8.5	1.00	4.01	5–5000
2-Butanol	0.77	n.d[b]	n.d	n.d	n.d	n.d	–
Ethyl butyrate	1.85	0.27	1.07	0.27	0.25	1.00	1
Butyl acetate	1.85	0.53	2.14	0.53	0.25	1.00	66
Hexanal	1.80	8	1	0.3	7.47	30.23	4.5–5
Isoamyl acetate	2.26	0.13	0.53	0.13	0.25	1.00	2
δ-3-Carene	4.61	0.034	0.034	0.009	0.99	3.79	n/a[c]
1-Butanol	0.84	270	540	136	0.50	1.98	500
β-Myrcene	4.88	0.034	0.034	0.009	0.99	3.80	13–15
α-Terpinene	4.75	0.14	0.14	0.036	1.00	3.78	n/a[c]
Amyl methyl ketone (2-heptanone)	1.73	0.13	2.13	0.26	0.06	0.50	140–3000
Limonene	4.83	0.034	0.034	0.009	1.00	3.81	10
3-Methyl-1-butanol (isoamyl alcohol)	1.26	68	270	69	0.25	0.97	250–300
(*E*)-2-Hexenal	1.58	16	8.6	2.1	1.86	7.52	17
Hexyl acetate	2.83	0.13	1.07	0.13	0.12	1.00	2
3-Hydroxy-2-butanone (acetoin)	-0.36	n.d	n.d	n.d	n.d	n.d	–
Octanal	2.78	8.3	0.3	0.07	30.90	125.26	0.7
(*E*)-2-Hexenyl acetate	2.61	0.13	0.53	0.13	0.25	1.00	n/a[c]
1-Hexanol	1.82	34	136	17	0.25	2.00	2500
(*Z*)-3-Hexen-1-ol	1.61	17	136	17	0.12	0.99	70
Hexyl butyrate	3.81	0.13	0.03	0.008	4.05	16.11	250
3-(Methylthio) propanal (methional)	0.41	4.2	2.2	0.5	1.92	7.78	0.2
Furfural	0.83	131	138	17	0.95	7.67	3000–23,000
Octyl acetate	3.81	0.07	0.03	0.008	2.01	8.00	12
Decanal	3.76	16	0.5	0.13	30.11	121.85	0.1–2
Benzaldehyde	1.71	33	8.6	1.1	3.85	31.13	350–3500
Linalool	3.38	0.13	1.07	0.27	0.12	0.49	6
Isobutyric acid	1.00	n.d	n.d	n.d	n.d	n.d	–
β-Caryophyllene	6.30	0.07	0.07	0.018	1.00	3.78	64
Acetophenone	1.67	1.1	4.5	1.1	0.25	1.01	65
Neral	3.45	0.15	0.61	0.15	0.25	1.00	30
α-Terpineol	3.33	0.14	1.11	0.27	0.12	0.52	300–350
Neryl acetate	4.48	0.03	0.07	0.017	0.50	2.01	2000–8500
Geranial	3.45	0.15	0.61	0.15	0.25	1.00	32
Valeric acid	1.56	286	n.d	275	n.d	1.04	3000
Geranyl acetate	4.48	0.03	0.07	0.017	0.50	2.01	9
1-Decanol	3.79	0.26	0.27	0.066	0.98	4.00	6–47
β-Citronellol	3.56	0.27	1.07	0.26	0.25	1.00	40
Nerol	3.47	0.27	2.20	0.54	0.12	0.51	300
β-Damascenone	4.21	0.016	0.07	0.016	0.25	1.00	0.002
Geraniol	3.47	0.27	2.13	0.54	0.13	0.50	40–75
2-Phenylethanol	1.57	17	68	17	0.25	1.00	750–1100
β-Ionone	4.29	0.008	0.07	0.017	0.13	0.50	0.007
Nonanoic acid	3.52	0.28	4.27	1.06	0.06	0.26	3000
Methyl anthranilate	2.26	0.54	1.07	0.53	0.50	1.01	3
Dodecanoic acid (lauric acid)	5.00	0.035	0.07	0.017	0.52	2.08	10,000

[a] Refs. [20] and [21].
[b] Not detected due to co-elution with octamethylcyclotetrasiloxane, a background peak from the PDMS coating on Twister™ stir bars.
[c] OTV data not available.

sample and the PDMS stir bar extraction phase) and of the diluted model wine (10 ml + 10 ml of analytical water – to reduce the possible matrix effects of ethanol) showed that the peak area responses of the aroma compounds were comparable.

On the other hand, the peak area responses obtained from the 20 ml model wine sample were in general double when compared to the lower volume and the diluted samples. This demonstrated that although performing SBSE on a smaller sample size, such as 10 ml, should theoretically increase the extraction recoveries of the aroma compounds by lowering the phase ratio, the overall decrease in analyte sensitivity outweighs this theoretical increase in recovery. This same conclusion also applies to the dilution of wine samples with water where the dilution effects outweighed the increase in analyte recoveries from reducing the effects of ethanol.

Changes in sensitivity of aroma compounds toward extraction from alcoholic beverages due to ethanol concentration has been demonstrated using SPME with PDMS as extraction phase in the analysis of vodkas and white rums at high % ethanol ranges [16]. Here the author found that, in general, higher responses of extracted aroma constituents were obtained in solutions of lower ethanol content. These results were expected because of the reduced solubility of the analytes in the "less organic" matrix. This effect was more pronounced for low molecular weight esters. A difference of 2% v/v from the normal ethanol strength (40% v/v) of spirits changed the absolute peak area responses by 10–37%. However, it was also demonstrated that unlike the absolute responses, the response ratios of all analytes to that of the internal standards used were not significantly affected by small variation in

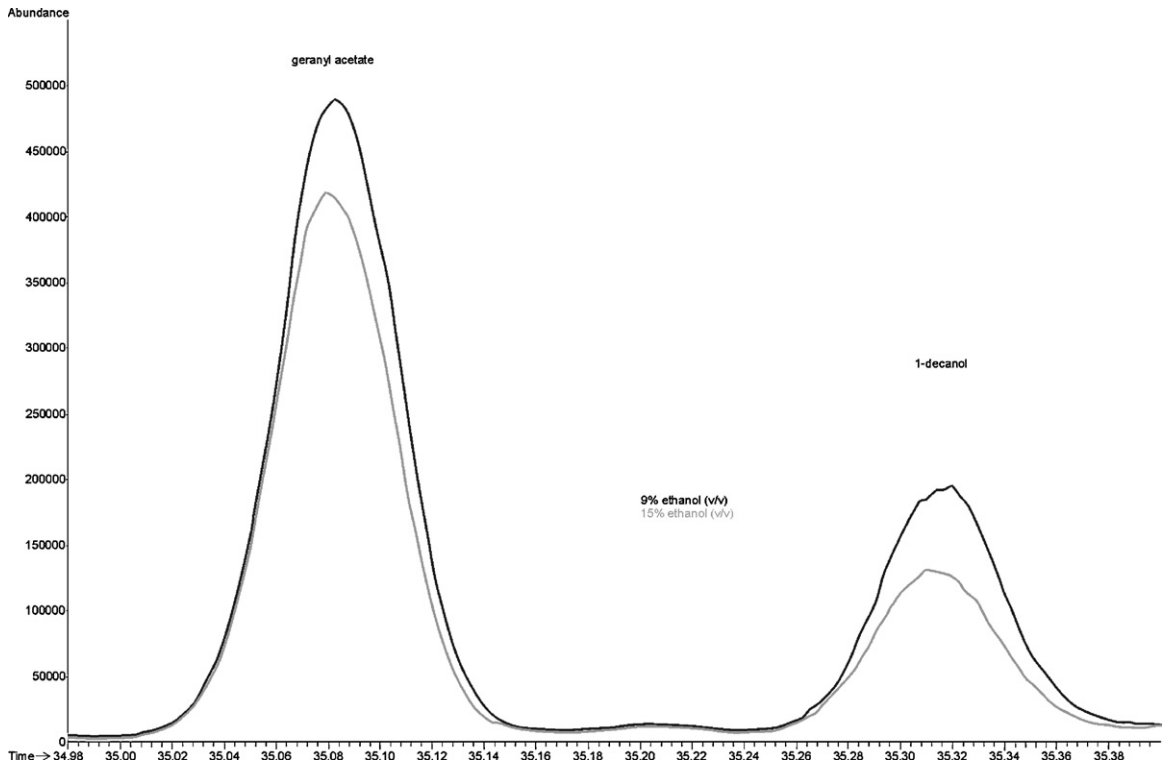

Fig. 1. Total ion chromatograms of geranyl acetate and 1-decanol extracted from synthetic wine of 9% and 15% ethanol content by volume.

ethanol content, and any deviations were largely due to experimental errors. These results suggest that the internal standards used were reasonably effective in correcting for the matrix effects caused by small variation in ethanol concentration.

3.4. Economical use of carrier gas and protection of sorbent phase and stationary phase

It is interesting to note the novel use of the Agilent GC "gas saver" in the 5:1 split ratio analysis of the model wine samples. The gas saver option is used in conventional split/splitless GC analysis to reduce the carrier gas flow from the split vent after a sample is transferred to the column. The Chemstation maintains column head pressure and the column flow rate, while purge and split vent flows decrease. This option is designed to save costly carrier gas such as Helium with a purity of 99.999%. When a "splitless" injection is performed on a standard split/splitless injector, the split vent flow is stopped on injection. This allows maximum sensitivity as the entire injected sample is theoretically transferred onto the capillary column. High flow rates such as 50–100 ml/min would usually then be applied back to the split vent after a period of 30–90 s to purge the split/splitless inlet of any residual sample volatiles/semi-volatiles. This is employed to sweep the inlet clean for subsequent injections to eliminate carry over (memory effects) from sample to sample.

When the TDS system uses low split flows (such as in this analysis case of 5 ml/min on transfer of the aroma compounds from the cryotrap to the column) the electronic pressure control pneumatics controlling the septum purge flow creates high air back-ground [28], which can be particularly damaging for GC stationary phases (the stir bar and the capillary column) from the increased levels of oxygen. Therefore, as it took 38 s for the CIS-4 to heat from −50 °C to 260 °C at 12 °C/s (CIS-4 has an "initial time" of 12 s where the PTV cryotrap maintains the initial temperature – the lowest setting recommended by Gerstel) and then it takes a further 26 s to heat to the upper temperature limit, it was demonstrated that a total time of 68 s was needed (with only 5 ml/min flow through the split vent) before introducing a higher flow rate to purge the inlet *via* the split vent. Thus, it was decided to use the Agilent gas saver technology to introduce a purge/split vent flow of 70 ml/min (i.e. identical to the desorption flow rate during analysis) to transfer aroma volatiles from the stir bar to the cryotrap) at 1.50 min. This also ensured that the increased air background was only occurring for the first 1.50 min of the analytical run when the column oven is still isothermal at 40 °C, hence minimising column damage.

Acknowledgements

The authors are indebted to Frutarom (UK) Ltd (originally Belmay (UK) Ltd) for funding and support of this PhD project. Financial support from KAIST is gratefully acknowledged.

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