Journal of Chromatography A, 1218 (2011) 504–517

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Development of a sensitive non-targeted method for characterizing the wine volatile profile using headspace solid-phase microextraction comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry

Anthony L. Robinson[a], Paul K. Boss[b], Hildegarde Heymann[c], Peter S. Solomon[d], Robert D. Trengove[a,*]

[a] Separation Science Laboratory, Murdoch University, South Street, Murdoch, WA 6150, Australia
[b] CSIRO Plant Industry, PO Box 350, Glen Osmond, SA 5064, Australia
[c] Department of Viticulture and Enology, University of California, Davis, CA 95616, USA
[d] Research School of Biology, The Australian National University, Canberra, ACT 0200, Australia

ARTICLE INFO

Article history:
Received 24 September 2010
Received in revised form 2 November 2010
Accepted 4 November 2010
Available online 11 November 2010

Keywords:
Comprehensive two-dimensional gas chromatography
GC × GC
HS-SPME
Wine
Volatile profiling
Aroma
Cabernet Sauvignon

ABSTRACT

Future understanding of differences in the composition and sensory attributes of wines require improved analytical methods which allow the monitoring of a large number of volatiles including those present at low concentrations. This study presents the optimization and application of a headspace solid-phase microextraction (HS-SPME) method for analysis of wine volatiles by comprehensive two-dimensional gas chromatography (GC × GC) time-of-flight mass spectrometry (TOFMS). This study demonstrates an important advancement in wine volatile analysis as the method allows for the simultaneous analysis of a significantly larger number of compounds found in the wine headspace compared to other current single dimensional GC–MS methodologies. The methodology allowed for the simultaneous analysis of over 350 different tentatively identified volatile and semi-volatile compounds found in the wine headspace. These included potent aroma compound classes such as monoterpenes, norisoprenoids, sesquiterpenes, and alkyl-methoxypyrazines which have been documented to contribute to wine aroma. It is intended that wine aroma research and wine sensory research will utilize this non-targeted method to assess compositional differences in the wine volatile profile.

© 2010 Published by Elsevier B.V.

1. Introduction

The fields of separation science and sensory science have advanced our knowledge of how volatile and semi-volatile compounds contribute to wine aroma [1,2]. With more than 800 aroma compounds reported in the volatile fraction of wine [3], it is well understood that the wine volatile profile is complex. Some studies have concluded that the vast majority of wine volatile compounds have little or no aroma activity and that specific aroma profiles can be explained by relatively few aroma compounds [4]. However, there is conflicting evidence about the complexity of the system given that odor mixtures have masking (modification of the perceived odor), counteraction (reduction of the odor intensity) [5], and synergistic (complementation or enhancement of the odor intensity) [6] effects which play an important role in defining the perceived aroma of wine [7,8]. It is thus important that grape and wine researchers develop the analytical capacity to measure as many volatiles as possible to enable better comparisons of effects of viticultural and winemaking studies and to identify candidate compounds that can be correlated with differences in the perceived aroma of wine.

The development of comprehensive two-dimensional gas chromatography (GC × GC) [9] has been followed by numerous reviews discussing the principals and experimental design of GC × GC [10–12]. These reviews have shown that GC × GC offers enhanced separation efficiency, reliability in qualitative and quantitative analysis, capability to detect low quantities, and information on the whole sample and its components. In more recent years, there has been a shift towards the use of this technique in the analysis of real-life samples including food and beverages, environmental, biological, and petrochemical [13].

A number of grape and wine profiling studies have used headspace solid-phase microextraction (HS-SPME) to better understand the role of various compounds in differentiating varieties, regions, and wine vintage [14–16] and the technique has been repeatedly documented as a sensitive, reproducible, automated method for pre-concentration of wine volatiles prior to analysis [17–19]. The combination of HS-SPME and GC × GC-TOFMS techniques has provided a major advantage in analyzing complex samples where the number of analytes may be large or the analytes

* Corresponding author. Tel.: +61 8 9360 7639; fax: +61 8 9360 6303.
E-mail address: R.Trengove@murdoch.edu.au (R.D. Trengove).

0021-9673/$ – see front matter © 2010 Published by Elsevier B.V.
doi:10.1016/j.chroma.2010.11.008

of interest are present at trace levels – as is the case with wine. A number of publications have emerged in the grape and wine field that have utilized HS-SPME and GC × GC as a technique [20–26]. However, the majority of studies have used the method for targeted analysis [20,22–24,26] with only two publications to date utilizing the technique for volatile profiling [21,25].

Rocha et al. [21] used GC × GC to analyze monoterpenes in grapes and identified 56 monoterpenes in the Fernão-Pires variety, of which 20 were reported for the first time in grapes. This highlighted the advantage that structured chromatographic separation can provide in compound classification and compound identity confirmation. There continues to be new aroma compound discoveries in the grape and wine research field with recent discoveries including (E)-1-(2,3,6-trimethylphenyl)buta-1,3-diene (TPB) [27] and 1(2H)-azulenone, 3,4,5,6,7,8-hexahydro-3,8-dimethyl-5-(1-methylethenyl)- ((−)-rotundone) [28]. It is anticipated that GC × GC will provide significant advantages in the identification of new and novel compounds which were previously unresolved using traditional one-dimensional chromatography.

A recent critical review [29] identified that future developments in understanding differences in the sensory attributes of wines will be due to: (1) development of improved and high throughput analytical methods that will allow monitoring of a large number of volatiles including those present at low concentrations; (2) improved understanding of the relationships between chemical composition and sensory perception, including an emphasis on the mechanisms of how odorants and matrix components interact chemically to impact odorant volatility and overall flavor perception of wines; and (3) multidisciplinary studies using genomic and proteomic techniques to understand flavor and aroma formation in the grape and during fermentation. The current study addresses the first recommendation from this publication and outlines a comprehensive analytical technique for the analysis of the wine volatile profile. The application of this technique to a small number of commercial wines clearly demonstrates that the optimized method can resolve and identify a large number of compounds and could be used in the future to differentiate wines based on their volatile profile.

2. Materials and methods

2.1. Samples

Method development was conducted using a young (<12 months old) commercially available Cabernet Sauvignon wine (~13.0% ethanol, v/v) from Australia. The wine was dispensed for use from a 2 L boxed wine bladder (cask) to minimize spoilage and oxidation during the course of analysis. Evaluation of the method was carried out using commercially available Cabernet Sauvignon wines with four wines from the 2005 vintage and one wine from the 2006 vintage representing four Western Australian Geographical Indications (GI, being the official delineation for wine regions within Australia). In all analysis 10 mL of wine was pipetted into the vial and sealed.

2.2. Analytical reagents and supplies

SPME fibers 1 cm and 2 cm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) 50/30 μm 23 Ga metal alloy were purchased from Supelco (Bellefonte, PA, USA). Prior to initial use, all new fibers were conditioned for 30 min at 270 °C as per the manufacturer's recommendations. Clear and amber glass, screw threaded, 20 mL headspace vials with magnetic screw caps and white PTFE/blue silicone (thickness 1.3 mm) septa were purchased from Alltech (Alltech Corp., Deerfield, IL, USA). Sodium chloride (NaCl) (AR Grade) was purchased from Merck Pty Ltd. (Kilsyth, Victoria, Australia) and was oven dried at 110 °C overnight before use. Methyl nonanoate (Quant Grade) was purchased from PolyScience (PolyScience, Niles, IL, USA). 2-Isobutyl-3-methoxypyrazine (99% pure) was purchased from Sigma (Sigma–Aldrich Corporation, St. Louis, MO, USA). Straight-chain alkanes (C_8–C_{20}) were purchased from Polyscience and Fluka (Sigma–Aldrich Corporation, St. Louis, MO, USA). HPLC grade n-pentane was purchased from Lab-Scan (Labscan Asia Co. Ltd., Patumwan, Bangkok, Thailand) and HPLC grade methanol was purchased from Burdick & Jackson (SK Chemicals, Ulsan, Korea). Inland 45 Vacuum pump fluid (pump oil) was purchased from Inland Vacuum Industries (Inland Vacuum Industries, Churchville, NY). Ultra-pure water was prepared using a Milli-Q water purification system to a resistivity of 18 MΩ cm (Millipore, Bedford, MA, USA).

2.3. Instrumentation

A CTC CombiPAL autosampler (CTC Analytics, Zwingen, Switzerland) with an agitator and SPME fiber conditioning station was used to extract the volatiles from the sample vial headspace. A LECO Pegasus® 4D GC × GC-TOFMS (LECO, St. Joseph, MI, USA) was used for all experiments. The GC primary oven was equipped with a 30 m Varian FactorFour™ VF-5MS capillary column, ID of 0.25 mm and a film thickness of 0.25 μm with a 10 m EZ-Guard™ column (Varian Inc., Walnut Creek, CA, USA). This was joined using a SilTite™ mini-union (SGE, Ringwood, Victoria, Australia) to a 1.65 m Varian FactorFour™ VF-17MS capillary column with an ID of 0.10 mm and a film thickness of 0.20 μm of which 1.44 m was coiled in the secondary oven. The non-polar and medium-polar column combination was chosen due to the low bleed characteristics of both the primary and secondary columns thus allowing for additional sensitivity for the analysis of trace analytes. A Supelco 0.75 mm ID SPME straight-through inlet liner (Bellefonte, PA, USA) was used for all injections. A High Pressure Merlin Microseal® (Bellefonte) was used for all 23 Ga SPME injections.

2.4. HS-SPME optimization

The following HS-SPME conditions were used during method development unless otherwise stated. Samples for HS-SPME method development were prepared in clear glass 20 mL headspace vials. Samples for GC × GC-TOFMS method development and evaluation were prepared in equivalent amber glass vials to prevent light degradation of alkyl-methoxypyrazines known to occur in Cabernet Sauvignon wines [30]. All samples were incubated at 30 °C with agitation at 500 rpm for 10 min prior to extraction at 250 rpm. DVB/CAR/PDMS SPME fibers were previously demonstrated to be suitable for non-targeted analysis of trace volatile and semi-volatile compounds in wine and were consequently used during this study [17,19]. The headspace was sampled using a 1 cm DVB/CAR/PDMS 50/30 μm metal alloy fiber for 60 min at 30 °C and desorbed in the GC inlet at 260 °C for 1 min. The fiber was then reconditioned using the fiber conditioning station for 5 min at 260 °C to prevent analyte carry over between samples. High purity (HP) nitrogen (Air Liquide, Australia) was passed over the fiber during reconditioning.

2.4.1. Desorption conditions

Fiber desorption times of 10, 20, 30, 40, 50, 60, 80, and 120 s were assessed at 250 °C. A second experiment assessed desorption temperatures of 230, 240, 250, 260, and 270 °C using a 60 s desorption time. Sample carry over was also assessed to determine the level of analytes not desorbed from the fiber prior to using the fiber conditioning station.

2.4.2. Salting out effect

Sodium chloride was added at concentrations of 0, 50, 100, 150, 200, 250, 300, 350, 400, 450, and 500 g L^{-1} to study the salting out effect.

2.4.3. Sample agitation

Agitation speeds of 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, and 750 rpm during extraction were examined. A second experiment was conducted to compare the effect of agitation on samples with and without salt. Extraction agitation speeds of 0, 300, 400, 500, 600, and 700 rpm were compared with samples that had been salted (300 g L^{-1}) and unsalted (0 g L^{-1}). All subsequent method development was conducted using an extraction agitation speed of 600 rpm as a compromise between extraction efficiency and fiber longevity.

2.4.4. Headspace extraction time and fiber length

Headspace extraction times of 30, 60, 90, 120, and 150 min were assessed comparing a 1 cm and a 2 cm length DVB/CAR/PDMS fiber.

2.4.5. Influence of sample incubation temperature

Samples were incubated at 30, 35, 40, 45, 50, 55 and 60 °C for 90 min and, after cooling to room temperature, were extracted for 90 min at 30 °C. These values were compared to a sample that remained at ambient temperature (20 °C).

2.5. Loading of internal standard into SPME fiber

Methyl nonanoate was chosen as an internal standard as it has not been previously reported in the literature as occurring in Cabernet Sauvignon wines and was not observed in the wine analyzed. The standard was loaded into the SPME fiber coating prior to the sample extraction step using methodology as previously described [19,31,32]. A 20 mL headspace vial containing 4 g of vacuum pump fluid and 20 μL of methyl nonanoate (1.1 g L^{-1} in HPLC grade methanol) was extracted for 5 min at 30 °C and 600 rpm.

2.6. Loading of retention index probes into SPME fiber

Retention index probes were loaded into the fiber coating after the internal standard as previously described [31]. A 20 mL headspace vial containing 1 mL MilliQ water and 10 μL of straight chain n-alkanes (C_8–C_{20}) in HPLC grade pentane was extracted under the same conditions as the internal standard [19]. Pentane was used as a solvent as hexane was found to overload the column and interfere with early eluting compounds. Alkanes were made up individually at varied concentrations to prevent the overloading of highly volatile low molecular weight probes and underloading of low volatility high molecular weight probes.

2.7. Chromatographic conditions

The injector was held at 260 °C in the splitless mode with a purge-off time of 1 min, a 50 mL min^{-1} split vent flow at 1 min and a gas saver flow of 20 mL min^{-1} at 3 min. Ultra high purity (UHP) helium (Air Liquide, Australia) was used as the carrier gas at a constant flow rate of 1.3 mL min^{-1}. The temperature program was 30 °C for 1 min, ramped at 3 °C min^{-1} to 240 °C, and held at 240 °C for 9 min. The secondary oven program was offset by +15 °C from the primary oven program and the modulator was offset by +30 °C from the primary oven. Single dimensional analysis acquired data at a rate of 10 scans s^{-1} as a compromise between sensitivity and facilitating sufficient peak deconvolution. For GC × GC mode, the data was acquired at a rate of 100 scans s^{-1} to accommodate the peak elution rate for modulated analytes. The transfer line and ion source were maintained at 250 °C and 200 °C, respectively, for both 1D and 2D experiments. The TOFMS detector was operated at 1750 V and collected masses between 35 and 350 amu.

2.8. Optimization of GC × GC parameters

Modulation periods were optimized by assessing modulation times of 4, 6, 8, 10, and 20 s with a secondary oven temperature offset of 15 °C to the primary oven. The secondary oven temperature offset was also assessed at +5, 10, 15, and 20 °C to the primary oven with a modulation period of 10 s.

2.9. Instrument control and data analysis software

Automated HS-SPME sample preparation was controlled using the PAL Cycle Composer with Macro Editor software Version 1.5.2. GC temperature programs, TOFMS data acquisition was controlled through the LECO ChromaTOF® software Version 3.32 optimized for Pegasus. Data analysis was conducted using LECO ChromaTOF® software Version 3.34 and used automated peak find and spectral deconvolution with a baseline offset of 0.5, auto data smoothing, and a signal to noise of 100. Results were matched against the NIST 2005 Mass Spectral Library using a forward search on all masses collected and calculated retention indices were compared to published retention indices for 5% phenyl polysilphenylene-siloxane capillary GC columns or equivalents [33,34]. All compounds tentatively assigned by the ChromaTOF software were manually assessed with respect to the mass spectral match and the assigned Unique mass which was used for quantification.

2.10. Statistical analysis

All statistical analysis was conducted using JMP version 7.0.1 (SAS Institute Inc., Cary, NC, USA). Figures and tables were generated using Microsoft Office Excel 2007 (Microsoft Corporation, Redmond, WA, USA).

2.11. SPME method optimization/data analysis

The relative responses of compounds, peak area of the unique ion expressed as a percentage of the maximum value recorded for the optimization parameter, were assessed in relation to the specific optimization parameter through hierarchical cluster analysis using a minimal variance algorithm [35]. Hierarchal cluster analysis is an unsupervised multivariate statistical technique which was employed to simplify the data analysis by clustering compounds that behaved in a similar manner. The cluster membership was then analyzed using a one-way analysis of variance (ANOVA) using a Tukey–Kramer HSD test to determine whether compound clusters responded differently to the specified optimization parameter. Cluster means ± standard error (SE) was then plotted against the optimization parameter with a second order line of best fit to depict the relative response of analytes to the optimization parameters.

3. Results and discussion

3.1. HS-SPME optimization

Although many compounds were identified, a representative selection of 25 target compounds, regarded as important contributors to wine aroma [1,2], were used for HS-SPME method optimization. The SPME optimization results are discussed with reference to Cluster membership of compounds listed in Table 1.

3.1.1. Desorption conditions

Fiber desorption temperature had a mixed influence on peak response. It was found that the peak area of compounds belonging

Table 1
Target compounds used for HS-SPME method optimization.

Compound	CAS	Unique ion (m/z)[a]	RT (s)	RI[b] (calc)	RI[c] (lit)	MS match	% RSD	Desorption clusters	Salting clusters	Agitation clusters	Time clusters	Incubation clusters
Ethyl propanoate	105-37-3	102	457.1	732	733	925	4%	C	E	H	J	L
Ethyl isobutyrate	97-62-1	116	557.1	769	756	784	8%	C	E	H	J	L
Ethyl butanoate	105-54-4	89	653.8	804	803	910	7%	C	E	H	J	L
Isohexanol	626-89-1	56	759.7	842	838	891	2%	C	D	H	K	L
Ethyl 2-methylbutyrate	7452-79-1	102	781.3	850	848	944	9%	C	E	H	J	L
Ethyl 3-methylbutyrate	108-64-5	88	794.2	855	852	870	8%	C	E	H	J	L
Ethyl pentanoate	539-82-2	88	929.7	903	898	886	5%	B	E	H	J	L
Methyl hexanoate	106-70-7	74	1000.0	926	923	891	4%	B	E	H	J	L
Hexyl acetate	142-92-7	84	1269.9	1014	1007	898	4%	B	E	H	J	L
p-Cymene	99-87-6	134	1311.1	1028	1026	845	5%	B	E	H	K	M
Eucalyptol	470-82-6	154	1337.3	1036	1033	852	2%	B	D	H	J	L
Benzyl Alcohol	100-51-6	108	1358.0	1043	1041	883	2%	A	D	G	I	L
Phenylacetaldehyde	122-78-1	120	1382.0	1051	1050	890	7%	A	D	G	K	L
Ethyl furoate	614-99-3	95	1396.1	1056	1056	890	6%	A	D	G	I	L
Terpinolene	586-62-9	93	1496.0	1088	1087	895	5%	B	D	G	K	M
Ethyl heptanoate	106-30-9	88	1527.0	1098	1093	905	8%	B	E	H	J	L
Linalool	78-70-6	93	1540.3	1103	1106	873	2%	B	D	H	J	N
α-Terpineol	98-55-5	136	1846.6	1210	1186	823	2%	B	D	F	I	L
2-Phenylethyl acetate	103-45-7	91	1992.5	1262	1256	906	2%	A	D	F	I	L
Vitispirane	65416-59-3	192	2062.6	1288	1272	961	8%	B	D	G	I	M
Methyl decanoate	110-42-9	74	2165.6	1326	1323	790	9%	A	E	G	K	N
(Z)-Oak lactone	55013-32-6	71	2174.3	1330	1340	870	4%	A	D	F	I	L
(Z)-β-Damascenone	23696-85-7	121	2266.7	1365	1367	812	3%	A	D	F	I	L
(E)-β-Damascenone	23726-93-4	121	2322.6	1386	1387	876	3%	B	D	F	I	L
Ethyl decanoate	110-38-3	101	2352.7	1397	1393	912	8%	A	E	H	K	N

[a] Used for peak area determination, identified as the unique ion by ChromaTOF data analysis.
[b] Retention indices calculated from C_8 to C_{20} n-alkanes.
[c] Retention indices reported in the literature for 5% phenyl polysilphenylene-siloxane capillary GC columns or equivalent [33,34].

to Cluster A increased from 48% to 87% of maximum between 230 and 260 °C, respectively (Fig. 1). However, compounds belonging to Clusters B and C increased and decreased by ~13% of maximum, respectively, within the same inlet temperature range. ANOVA indicated that there was no significant difference in the cluster means between 260 and 270 °C for all compound clusters, thus subsequent analysis was conducted at 260 °C. Analyte carry over declined with increasing desorption temperature, with all trace compounds being below detection threshold and the higher abundant compounds declining to less than 5% of the analyzed peak area (data not presented). A 5 min conditioning step at 270 °C prevented any carry over effects.

3.1.2. Salting out effect

The standard addition of 300 g L^{-1} sodium chloride to a wine was selected, given that it covers the saturation range of sodium chloride for the majority of table wines. The resulting salting out, or Setschenow effect [36], led to an increase in peak area for all compounds analyzed. ANOVA indicated that increasing concentrations of salt above 300 and 200 g L^{-1} for compounds in Clusters D and E, respectively, did not result in a statistically significant change. Compounds belonging to Cluster D increased from 20% to 88% of maximum at 300 g L^{-1} however compounds belonging to Cluster E increased from 53% to 91% of maximum at 200 g L^{-1} (Fig. 2).

Compounds belonging to Cluster D had a range of different functionalities while compounds belonging to Cluster E were typically ethyl and methyl esters with the exception of p-cymene. This is consistent with pharmaceutical research relating the salting out effect in a sodium chloride solution to molar volume, aqueous solubility, and the octanol–water partition coefficient ($K_{o/w}$) [37,38]. Further, Ferreira et al. [39] observed that the ethyl esters had particularly high gas-liquid partition coefficient (GLPC) values and suggested

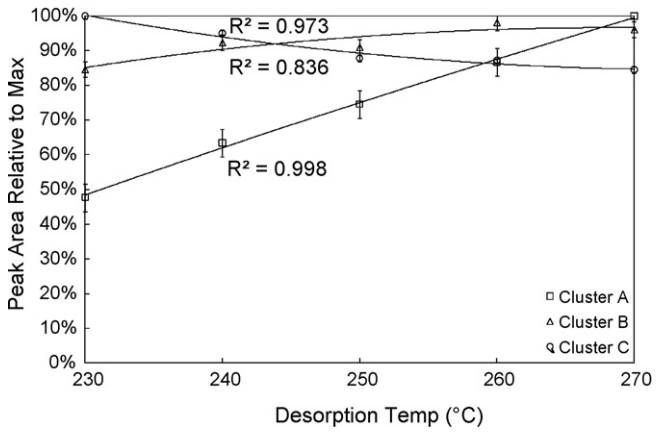

Fig. 1. Influence of inlet desorption temperature on the relative peak area response. Relative peak area is expressed as a percentage of the maximum value recorded. Data points represent the mean (±SE) of compounds belonging to Clusters A, B and C.

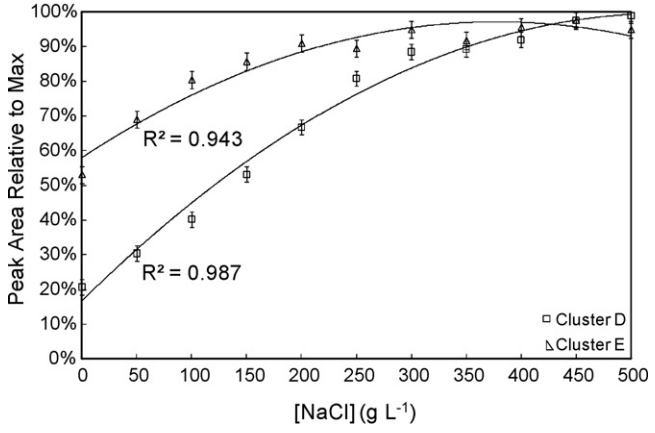

Fig. 2. Influence of sodium chloride concentration on the relative peak area response. Relative peak area is expressed as a percentage of the maximum value recorded. Data points represent the mean (±SE) of compounds belonging to Clusters D and E.

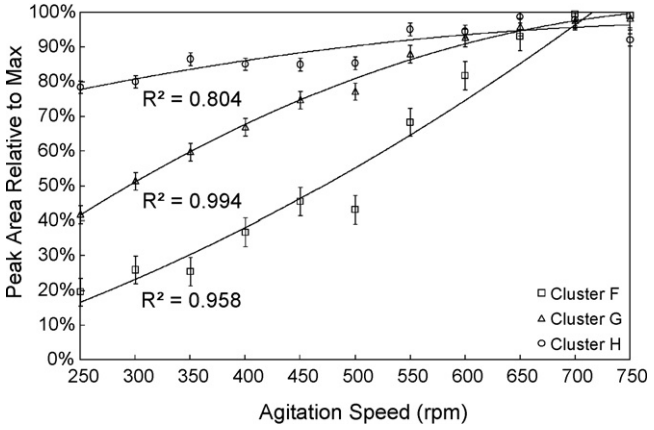

Fig. 3. Influence of sampling agitation speed on the relative peak area response. Relative peak area is expressed as a percentage of the maximum value recorded. Data points represent the mean (±SE) of compounds belonging to Clusters F, G and H.

that their behavior could be best explained firstly by the functionality, or polarity, and then by their intrinsic volatility.

3.1.3. Sample agitation

ANOVA indicated that there was no significant difference in the cluster means between 600 rpm and subsequent agitation speeds for all three cluster groups. Compounds belonging to Cluster F increased from 20% to 82% of maximum between 250 and 600 rpm, respectively (Fig. 3). Compounds belonging to Cluster G and H increased 46% and 17% of maximum between 250 and 600 rpm, respectively.

Compounds tended to cluster according to molecular weight and vapor pressure. That is, compounds belonging to Cluster H had lower molecular weights with higher vapor pressures, while compounds belonging to Cluster F were characterized by higher molecular weight and lower vapor pressures and compounds belonging to Cluster G had intermediate molecular weight and vapor pressures compared to compounds belonging to Clusters F and H. The impact of molecular weight is consistent with the diffusion dependence on this property.

3.1.4. Salt and agitation interactions

Previous studies have demonstrated that the new-generation super elastic metal alloy SPME fibers are capable of carrying out several hundred extraction cycles [32] without showing any significant loss in sensitivity, with one study conducting more than 600 cycles using a single fiber [16]. However, each extraction in the studies by Setkova et al. [16,32] exposed the SPME fiber to agitation stress for 5 min at 500 rpm per extraction which would equate to 50 h of agitation stress. In this study we found that extreme agitation caused scoring of the SPME needle and eventually damaged the fiber, thus an agitation speed of 600 rpm was selected as a compromise to optimize sensitivity while maintaining the fiber lifetime.

3.1.5. Headspace extraction time and fiber length

The fiber length by extraction time interaction was significant with the 2 cm fiber compared with a 1 cm fiber providing greater peak area values for all compounds (Fig. 4(a) and (b)). Compounds belonging to Clusters I and K increased with increasing extraction time while compounds belonging to Cluster J remained constant with respect to extraction time. However, ANOVA indicated that the compounds belonging to Cluster J at 120 min increased from 59% to 98% of maximum with the increase in fiber length from 1 to 2 cm. Compounds belonging to Clusters I and K were not significantly different after 120 and 90 min, respectively. A maximum

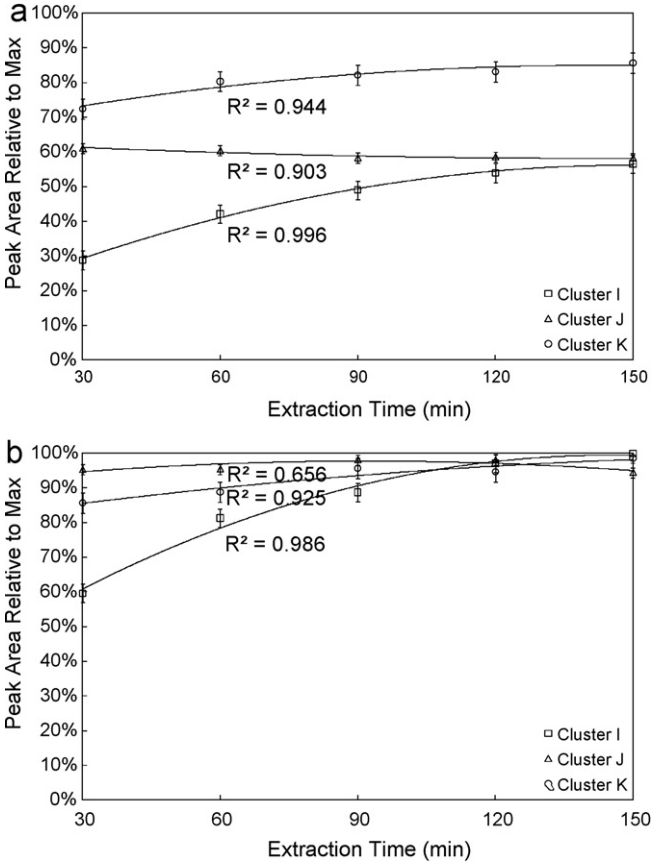

Fig. 4. Influence of sampling time on the relative peak area response using (a) 1 cm and (b) 2 cm fiber lengths. Relative peak area is expressed as a percentage of the maximum value recorded. Data points represent the mean (±SE) of compounds belonging to Clusters I, J and K.

relative peak area was achieved for all compounds after 120 min of extraction using a 2 cm fiber length.

3.1.6. Influence of sample incubation temperature

A previous study correlated the presence of artifacts with HS-SPME extraction temperature in honey samples [40] and this phenomenon, was investigated for wines by incubating samples from 30 to 60 °C for 90 min as described previously. The results of the analysis are shown in Fig. 5. ANOVA indicated that the abundance of compounds within Clusters L and N declined

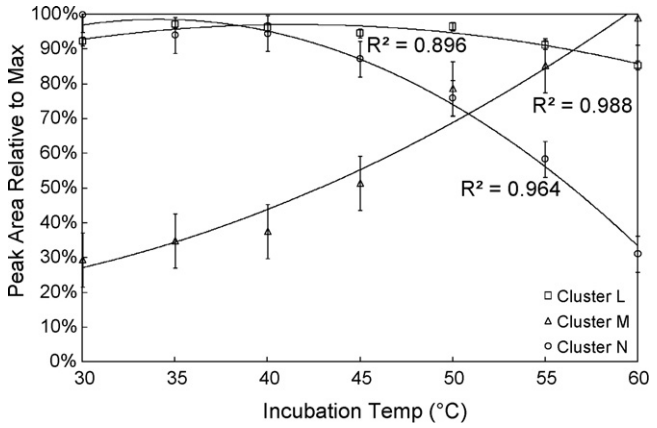

Fig. 5. Influence of incubation temperature on the relative peak area response. Relative peak area is expressed as a percentage of the maximum value recorded. Data points represent the mean (±SE) of compounds belonging to Clusters L, M and N.

Table 2
Optimized HS-SPME/GC × GC-TOFMS conditions used for the analysis of five commercial Cabernet Sauvignon Wines from Western Australia.

HS-SPME	
HS vial	20 mL amber headspace vial
Sample volume	10 mL wine
Salt addition	300 g L^{-1}
SPME fiber	DVB/CAR/PDMS 50/30 μm, 2 cm, 23 Ga metal alloy
Incubation conditions	30 °C/600 rpm/5 min
Extraction conditions	30 °C/600 rpm/120 min
Desorption conditions	260 °C/1 min
Fiber bake-out conditions	270 °C/5 min
GC × GC	
Injector mode	Splitless
1 GC column	VF-5MS (30 m × 0.25 mm I.D. × 0.25 μm and 10 m EZ-Guard)
2 GC column	VF-17MS (1.65 m × 0.10 mm I.D. × 0.20 μm)
Carrier gas	UHP helium
Gas flow	Constant flow, 1.3 mL min^{-1}
GC oven program	30 °C (1 min)/3 °C min^{-1} to 240 °C (9 min)
Secondary oven offset	+5 °C
Modulation period	6 s
Transfer line temperature	250 °C
TOFMS	
Detector voltage	1750 V
Data acquisition rate	100 scans s^{-1}
Mass range	35–350 amu
Ion source temperature	200 °C

significantly at incubation temperatures above 50 °C and 45 °C, respectively, while compounds belonging to Cluster M increased significantly at incubation temperatures above 40 °C. Linalool and ethyl decanoate (Cluster N) showed significant declines in concentration and reflected changes in a number of other compounds including methyl decanoate.

Vitispirane, p-cymene and terpinolene represent a much larger set of compounds, including 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN), and dehydroxylinalool oxide, that changed more dramatically with respect to incubation temperature. Silva Ferreira et al. have studied the formation of Vitispirane and TDN with respect to temperature, time, SO_2 concentration, and dissolved oxygen concentration [41,42]. It was shown that temperature and pH were particularly important to the formation of both Vitispirane and TDN [42]. Previous research has indicated that both Vitispirane and TDN are generated from multiple glycosylated precursors that are hydrolyzed under acidic conditions which can be accelerated by elevated temperature [43,44]. It also followed that the degradation of linalool and formation of linalool oxides was accelerated at 45 °C compared to 15 °C temperatures [41].

This is the first study that has documented the formation of artifacts in wine through the use of increased temperature during the SPME incubation step. Given that products were generated and lost under elevated temperature conditions, the lowest controlled temperature available, 30 °C, was chosen as the optimum temperature for incubation and extraction of the sample.

3.2. Repeatability of SPME method

Six replicate extractions of the cask wine were analyzed with the optimized HS-SPME method (Table 2). The internal standard, methyl nonanoate, and retention index probes were loaded into the fiber prior to sample extraction which made their response independent of the sample matrix as previously demonstrated [19,31,32]. RSD values were calculated using the peak area values normalized against the in-fiber internal standard and are presented in Table 1. RSDs of the normalized peak area ranged from 2% to 9% which was comparable to previous HS-SPME studies [17–19].

3.3. Optimization of GC × GC parameters

The objective of coupling HS-SPME to GC × GC-TOFMS was to analyze a substantial number of compounds with gains in sensitivity and resolution from GC × GC modulation coupled to gains in sensitivity and selectivity from HS-SPME. In comprehensive two-dimensional gas chromatography, samples are resolved through two chromatographic separations in series. This process is aided by a modulator which periodically collects, focuses, and reintroduces the eluent at the end of the primary column into the secondary column where it undergoes an isothermal separation before reaching the detector. The major advantage of this process is that the first dimension separation is maintained while allowing additional separation in the second dimension [12]. Parameters controlling the second dimension of chromatography were investigated to determine their influence on resolution.

In order to preserve the primary dimension separation the modulator should sample the first dimension as frequently as possible [45]. To better accomplish this, it is understood that temperature programming in GC × GC is usually at a lower rate than in one-dimensional gas chromatography, i.e. at 2–3 °C min^{-1} [13]. The resolution of two closely eluting compounds, TDN and (Z)-β-damascenone, were examined at varying modulation times. These two compounds were selected as an example as (E)-β-damascenone is well recognized as a potent aroma compound in wine [7] while the (Z)- isomer of β-damascenone, which is present at much lower concentrations, has rarely been identified and reported in wine related studies. Fig. 6 shows that the shorter modulation time of six seconds resolved TDN and (Z)-β-damascenone, while 10 and 20 s modulation times caused a loss in primary dimension separation with both compounds recombined in the modulator [46]. These two compounds were resolved in the first dimension ($R_{S1} \approx 1.1$) but not well resolved in the second dimension ($R_{S2} \approx 0.1$), at the natural concentrations found in the cask wine used.

Literature typically suggests that any first dimension peak should be sampled by the modulator at least three times when the sampling is in-phase and four times when the sampling is 180° out-of-phase [10,47]. With a modulation period of 6 s the majority of peaks were sampled three times or more. Attempting to optimize the modulation phase or peak pulse profiles for all compounds in a real sample is a complex process due to errors associated with the summation of multiple modulated peaks and errors due to shifts in the phase of the primary peak relative to the modulation period [48].

In practice, the sample rate in the first dimension is limited by the duration of the second dimension separation. To maintain the ordered structure of the chromatogram, compounds should elute within the modulation cycle to prevent compounds from different modulation cycles co-eluting [11]. Decreasing the modulation time to five seconds or less produced a wrap-around effect for a number of substituted benzene compounds and a number of γ- and δ-lactones (data not presented). A comparison of secondary oven temperature offsets showed that higher temperature offsets reduced the second dimension retention time. Increasing the secondary temperature offset from 5 to 20 °C resulted in a 15% reduction in secondary dimension retention time with each 5 °C increment for a number of compounds including the lactones (data not shown). This was accompanied by a reduction in peak width and second dimension resolution. A 6 s modulation time with a 5 °C secondary oven temperature offset was chosen to be a suitable compromise as it maintained the first dimension separation, maximized the second dimension resolution, and produced a minimal

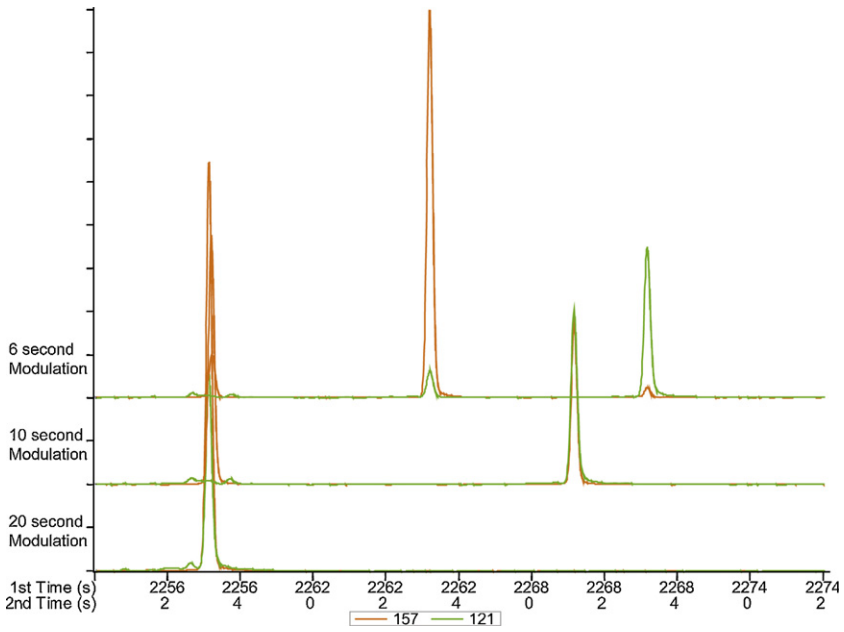

Fig. 6. Influence of 6, 10 and 20 s modulation times on the second dimension separation of TDN (*m*/*z* 157) and (Z)-β-damascenone (*m*/*z* 121). Note with increasing modulation time that the first dimension separation is compromised.

wrap-around effect for compounds that were late to elute from the second dimension. As an example, Fig. 7 presents a typical contour plot of a HS-SPME/GC × GC-TOFMS chromatogram from a Cabernet Sauvignon wine.

3.4. Sensitivity and deconvolution using GC × GC and ChromaTOF

Ryan et al. previously demonstrated that GC × GC could be used as a sensitive technique for the analysis of alkyl methoxypyrazines in wines [20]. A 2006 vintage Cabernet Sauvignon from Western Australia was anecdotally considered to have a bell-pepper aroma which has previously been associated with the potent aroma compound 2-isobutyl-3-methoxypyrazine (IBMP) [30]. The 2006 vintage wine was analyzed using the optimized method and IBMP was matched to a peak using the deconvoluted mass spectrum and retention index. However, the qualifier ions, 94 and 151 which are 24% and 18% of the base peak, respectively, were common to two closely eluting compounds. To confirm the retention time and mass spectral match of the compound the same wine was spiked with approximately 4 ng L^{-1} IBMP. The first and second dimension retention times were an exact match with a signal to noise of 209 and 407 for the wine and spiked wine, respectively (Fig. 8). This confirmed that the optimized methodology was sensitive enough to analyze the potent odor compound IBMP at ppt concentration levels at and below odor threshold for this compound [20,30].

3.5. Wine volatile profile compound identification

Five commercial Cabernet Sauvignon wines from Western Australia were analyzed using the optimized HS-SPME/GC × GC-TOFMS method described in Table 2. Compounds were compared against the NIST 2005 Mass Spectral Library and published retention indices [33,34] for identity confirmation, Table 3. Metabolite profiling by GC–MS and subsequent statistical analysis relies on efficient data-processing procedures. The minimum reporting requirements for chemical analysis have recently been suggested

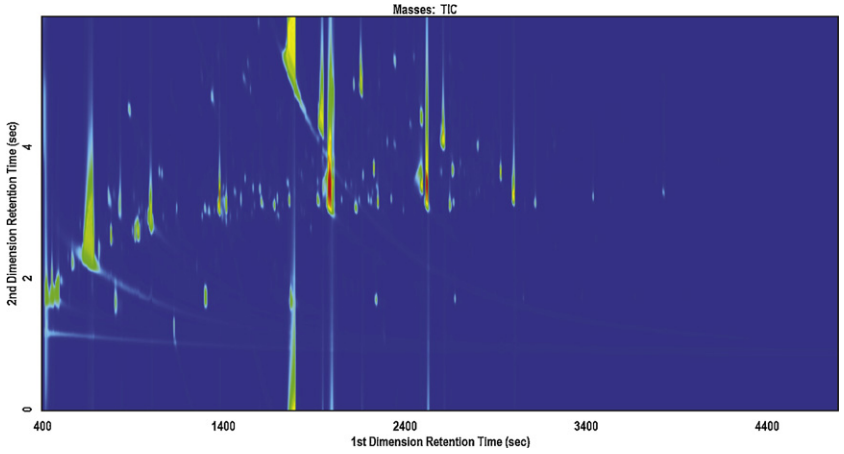

Fig. 7. Typical contour plot of a HS-SPME/GC × GC-TOFMS chromatogram (TIC) demonstrating the separation of volatile compounds isolated from the headspace of a Cabernet Sauvignon wine. The color gradient reflects the intensity of the TOFMS signal (*Z*-axis) from low (blue) to high (red). Note that a substantial number of trace volatile compounds are not visible in this chromatogram due to the abundant esters dominating the *Z*-axis of the plot.

Table 3
Compound names, CAS numbers, unique masses, mean mass spectral match quality, retention times, and retention indices for compounds analyzed by GC × GC-TOFMS based on MS and RI matches for five commercial Cabernet Sauvignon wines from Western Australia.

Peak#	Compound	CAS	Unique mass[a]	MS match	1 RT(s)	2 RT(s)	RI[b] (calc)	RI[c] (lit)
1	Isobutyl alcohol	78-83-1	74	845	348	1.703	695	650
2	1-Butanol	71-36-3	56	823	396	1.819	711	662
3	1-Penten-3-ol	616-25-1	57	846	420	1.838	720	684
4	2-Ethylfuran	3208-16-0	81	767	432	1.838	724	720
5	1-Propene, 1-(methylthio)-, (E)-	42848-06-6	73	801	432	1.939	724	726
6	2,3-Pentanedione	600-14-6	57	800	432	2.088	724	697
7	2,5-Dimethylfuran	625-86-5	96	788	444	1.881	729	728
8	Ethyl propanoate	105-37-3	102	918	456	2.034	733	726
9	Propyl acetate	109-60-4	43	917	462	2.031	735	728
10	Acetal	105-57-7	47	812	486	1.786	744	726
11	2,4,5-Trimethyl-1,3-dioxolane	3299-32-9	101	838	486	1.938	744	735
12	Acetoin	513-86-0	88	819	486	2.662	745	743
13	Ethyl isobutyrate	97-62-1	116	841	552	2.147	768	756
14	Isobutyric acid	79-31-2	73	852	567	2.815	773	775
15	Toluene	108-88-3	91	919	570	2.404	774	771
16	2-Methylthiophene	554-14-3	97	831	582	2.676	778	775
17	Isobutyl acetate	110-19-0	56	881	588	2.223	781	780
18	3-Methylthiophene	616-44-4	98	778	600	2.744	785	786
19	Diethyl carbonate	105-58-8	91	854	618	2.762	792	765
20	2,3-Butanediol	513-85-9	47	899	636	3.304	798	789
21	Butanoic acid	107-92-6	60	726	636	3.365	798	789
22	Octane[d]	111-65-9	85	735	642	1.545	800	800
23	2-Ethyl-5-methylfuran	1703-52-2	95	775	642	2.360	800	802
24	Ethyl butanoate	105-54-4	89	913	648	2.470	803	803
25	Hexanal	66-25-1	82	682	654	2.662	805	804
26	Dibromochloromethane	124-48-1	129	849	654	3.402	806	800
27	Tetrachloroethylene	127-18-4	166	888	660	2.439	807	815
28	Butyl acetate	123-86-4	61	882	684	2.491	816	813
29	Ethyl lactate	97-64-3	75	795	690	3.068	818	815
30	1,3-Octadiene	1002-33-1	54	902	708	1.979	824	827
31	Methyl ethyl disulfide	20333-39-5	108	711	744	3.147	837	846
32	Furfural	98-01-1	96	930	744	4.513	838	835
33	Ethyl crotonate	10544-63-5	69	898	768	3.000	847	834
34	Chlorobenzene	108-90-7	112	836	774	3.190	848	852
35	Ethyl 2-methylbutyrate	7452-79-1	102	927	780	2.493	850	848
36	Isohexanol	626-89-1	56	812	780	2.684	851	838
37	S-Methylmercaptoethanol	5271-38-5	61	834	780	4.121	851	838
38	Isovaleric acid	503-74-2	60	843	786	3.126	853	839
39	Ethyl isovalerate	108-64-5	88	890	792	2.529	855	852
40	3-Hexen-1-ol, (E)-	928-97-2	67	851	792	2.936	855	853
41	3-Hexen-1-ol, (Z)-	928-96-1	67	939	804	2.932	860	860
42	Ethylbenzene	100-41-4	91	931	810	2.859	861	866
43	2-Furanmethanol	98-00-0	98	878	810	4.047	862	866
44	2-Methylbutanoic acid	116-53-0	74	903	816	3.196	864	850
45	2-Ethylthiophene	872-55-9	97	779	822	3.129	866	871
46	m-Xylene	108-38-3	91	907	834	2.842	870	874
47	1-Hexanol	111-27-3	56	893	840	2.821	873	863
48	Isoamyl acetate	123-92-2	70	797	858	2.707	879	876
49	3,4-Dimethylthiophene	632-15-5	111	804	858	3.291	879	887
50	2-Methylbutyl acetate	624-41-9	70	810	864	2.658	880	875
51	2-Butylfuran	4466-24-4	81	710	894	2.593	892	894
52	2-Heptanone	110-43-0	58	894	894	2.960	892	889
53	o-Xylene	95-47-6	91	901	900	3.109	894	894
54	Styrene	100-42-5	104	895	900	3.380	894	897
55	Nonane[d]	111-84-2	57	897	918	1.737	900	900
56	Propyl butanoate	105-66-8	71	801	918	2.715	900	896
57	Ethyl pentanoate	539-82-2	88	906	924	2.746	903	898
58	2-Heptanol	543-49-7	45	876	936	2.601	906	901
59	Heptanal	111-71-7	86	857	936	2.911	906	900
60	2-Acetylfuran	1192-62-7	95	917	960	4.740	915	914
61	Isobutyl isobutyrate	97-85-8	71	823	966	2.442	916	906
62	Pentyl acetate	628-63-7	70	828	966	2.769	916	916
63	γ-Butyrolactone	96-48-0	86	945	978	1.420	920	915
64	Anisole	100-66-3	108	813	978	3.921	921	920
65	Methyl hexanoate	106-70-7	74	893	996	2.840	926	923
66	Cumene	98-82-8	105	798	996	2.953	925	924
67	Ethyl tiglate	5837-78-5	113	820	1038	3.207	940	939
68	Ethyl 3-hydroxybutanoate	5405-41-4	71	875	1038	3.644	940	945
69	Camphene	79-92-5	93	746	1074	2.458	951	961
70	Propyl isovalerate	557-00-6	85	835	1074	2.634	951	949
71	Propylbenzene	103-65-1	91	884	1086	3.031	955	957
72	Isobutyl butanoate	539-90-2	71	850	1092	2.632	957	955
73	Ethyl 3-methylpentanoate	5870-68-8	88	794	1098	2.717	960	960
74	m-Ethyl toluene	620-14-4	120	883	1110	3.073	964	969
75	Ethyl isohexanoate	25415-67-2	88	883	1122	2.745	967	969

Table 3 (*Continued*)

Peak#	Compound	CAS	Unique mass[a]	MS match	1 RT(s)	2 RT(s)	RI[b] (calc)	RI[c] (lit)
76	Ethyl 2-hydroxyisovalerate	2441-06-7	104	822	1122	3.112	967	987
77	Benzaldehyde	100-52-7	106	903	1122	4.959	968	969
78	5-Methylfurfural	620-02-0	110	893	1122	5.159	968	964
79	Dehydroxylinalool oxide A	7392-19-0	139	840	1134	2.506	971	971
80	Isoamyl propanoate	105-68-0	57	880	1134	2.744	971	969
81	1-Heptanol	111-70-6	56	891	1140	2.949	973	970
82	Dimethyl trisulfide	3658-80-8	126	871	1140	4.615	973	982
83	Methyl furoate	611-13-2	95	915	1158	4.970	979	985
84	o-Ethyltoluene	611-14-3	105	877	1164	3.278	980	988
85	Octen-3-ol	3391-86-4	57	843	1170	2.845	983	986
86	α-Methylstyrene	98-83-9	118	836	1176	3.517	985	988
87	Ethyl (methylthio)acetate	4455-13-4	134	739	1182	4.313	987	990
88	Methionol	505-10-2	106	918	1182	4.733	987	982
89	3-Octanone	106-68-3	99	842	1188	3.019	988	989
90	Methyl heptenone	409-02-9	108	740	1188	3.417	988	987
91	β-Myrcene	123-35-3	93	874	1194	2.461	990	991
92	2-Amylfuran	3777-69-3	81	800	1194	2.773	991	993
93	2-Octanone	111-13-7	58	781	1200	3.099	993	990
94	2-Carene	554-61-0	121	737	1212	2.685	997	1001
95	6-Methyl-5-hepten-2-ol	1569-60-4	95	842	1212	3.022	997	993
96	Pseudocumene	95-63-6	105	933	1212	3.217	997	1000
97	Phenol	108-95-2	94	803	1212	4.474	996	979
98	2-Methylthiolan-3-one	13679-85-1	116	849	1212	5.323	997	994
99	Decane[d]	124-18-5	43	896	1224	1.899	1000	1000
100	Benzofuran	271-89-6	118	848	1224	4.486	1001	1007
101	(Z)-3-Hexenyl acetate	3681-71-8	67	814	1236	3.120	1004	1006
102	Octanal	124-13-0	84	818	1242	3.080	1006	1003
103	α-Phellandrene	99-83-2	136	682	1248	2.624	1009	1005
104	Ethyl-3-hexanoate	2396-83-0	142	879	1248	3.213	1008	1007
105	α-Thiophenecarboxaldehyde	98-03-3	111	912	1254	0.076	1009	1010
106	m-Dichlorobenzene	541-73-1	146	796	1254	3.840	1010	1022
107	Ethylfurylketone	3194-15-8	95	851	1254	4.794	1011	1008
108	1-Methyl-2-formylpyrrole	1192-58-1	109	814	1254	5.530	1011	1010
109	Isoamyl isobutyrate	2050-01-3	89	844	1266	2.655	1014	1018
110	Hexyl acetate	142-92-7	84	894	1266	2.923	1014	1007
111	Hexanoic acid	142-62-1	60	910	1266	3.442	1015	978
112	α-Terpinene	99-86-5	93	854	1278	2.671	1019	1018
113	Isocineole	470-67-7	111	828	1278	2.794	1018	1016
114	Benzyl chloride	100-44-7	91	801	1278	4.542	1019	1023
115	p-Dichlorobenzene	106-46-7	146	892	1284	3.957	1020	1015
116	(S)-3-Ethyl-4-methylpentanol	0-00-0	84	883	1296	3.017	1024	1020
117	Hemimellitene	526-73-8	105	932	1296	3.527	1024	1033
118	p-Cymene	99-87-6	134	859	1308	3.100	1027	1026
119	Limonene	5989-27-5	68	884	1320	2.670	1032	1031
120	2-Ethyl hexanol	104-76-7	57	890	1320	2.883	1032	1030
121	Eucalyptol	470-82-6	108	869	1332	2.957	1036	1033
122	(Z)-Ocimene	3338-55-4	92	847	1338	2.661	1038	1040
123	Indane	496-11-7	117	862	1338	3.929	1038	1048
124	2-Acetyl-5-methylfuran	1193-79-9	109	849	1338	5.100	1039	1042
125	2,2,6-Trimethylcyclohexanone	2408-37-9	82	883	1344	3.464	1039	1035
126	Benzyl alcohol	100-51-6	108	916	1356	5.069	1044	1041
127	Lavander lactone	1073-11-6	111	755	1356	5.691	1045	1041
128	Ocimene quintoxide	7416-35-5	139	712	1362	2.828	1046	1049
129	Ethyl 2-hexenoate	27829-72-7	99	922	1362	3.371	1046	1036
130	(E)-Ocimene	3779-61-1	93	847	1368	2.680	1047	1051
131	3-Nonen-5-one	82456-34-6	83	801	1374	3.095	1050	1051
132	Salicylaldehyde	90-02-8	122	812	1374	5.092	1051	1057
133	Phenylacetaldehyde	122-78-1	120	900	1374	5.231	1051	1050
134	m-Propyltoluene	1074-43-7	105	850	1386	3.122	1053	1052
135	Ethyl furoate	614-99-3	95	908	1392	4.819	1056	1056
136	Isoamyl butyrate	106-27-4	71	892	1398	2.806	1057	1054
137	Butylbenzene	104-51-8	91	835	1398	3.185	1058	1058
138	Ethyl 2-hydroxy-4-methylpentanoate	10348-47-7	69	914	1404	3.224	1059	1060
139	γ-Hexalactone	695-06-7	85	876	1410	0.202	1060	1063
140	γ-Terpinene	99-85-4	93	817	1410	2.855	1061	1062
141	o-Cresol	95-48-7	108	851	1434	4.491	1069	1077
142	Diethyl malonate	105-53-3	115	862	1434	4.382	1070	1069
143	Ethyl 5-methylhexanoate	10236-10-9	88	722	1440	2.899	1071	1072
144	Acetophenone	98-86-2	105	926	1440	5.269	1072	1076
145	1-Octanol	111-87-5	56	904	1452	3.032	1075	1080
146	p-Tolualdehyde	104-87-0	119	835	1452	4.992	1075	1079
147	2-Ethyl-p-xylene	1758-88-9	119	673	1458	3.320	1078	1077
148	Terpinolene	586-62-9	93	915	1488	2.982	1087	1087
149	4-Ethyl-o-xylene	934-80-5	119	856	1488	3.348	1087	1093
150	p-Cresol	106-44-5	107	869	1500	4.501	1091	1077
151	Guaiacol	90-05-1	109	896	1500	5.055	1092	1102
152	2-Nonanone	821-55-6	58	793	1506	3.153	1093	1092

Table 3 (*Continued*)

Peak#	Compound	CAS	Unique mass[a]	MS match	1 RT(s)	2 RT(s)	RI[b] (calc)	RI[c] (lit)
153	Dehydro-p-cymene	1195-32-0	117	927	1506	3.585	1093	1091
154	Propyl hexanoate	626-77-7	99	899	1512	2.909	1095	1079
155	Ethyl heptanoate	106-30-9	88	914	1524	2.932	1098	1093
156	Methyl benzoate	93-58-3	105	901	1524	4.768	1099	1100
157	Undecane[d]	1120-21-4	57	889	1530	1.947	1099	1100
158	Isopentyl 2-methylbutanoate	27625-35-0	85	872	1530	2.703	1100	1100
159	Ethyl sorbate	2396-84-1	140	854	1530	3.825	1101	1103
160	Linalool	78-70-6	93	893	1536	3.031	1103	1106
161	Ethyl methylthiopropanoate	13327-56-5	74	913	1536	4.373	1103	1098
162	2-Nonanol	628-99-9	45	906	1542	2.803	1105	1098
163	Isopentyl isovalerate	659-70-1	85	877	1548	2.707	1107	1105
164	Nonanal	124-19-6	95	893	1548	3.120	1107	1106
165	Heptyl acetate	112-06-1	43	862	1566	2.931	1113	1115
166	(Z)-Rose oxide	16409-43-1	139	830	1566	3.074	1113	1112
167	2-Methylcumarone	4265-25-2	131	887	1566	4.449	1113	1109
168	1,3,8-p-Menthatriene	21195-59-5	134	793	1572	3.406	1115	1111
169	α-Cyclocitral	432-24-6	81	772	1596	3.605	1124	1116
170	Methyl octanoate	111-11-5	127	879	1602	3.002	1126	1129
171	2-Ethylhexanoic acid	149-57-5	88	721	1620	3.300	1132	1128
172	α-Isophoron	78-59-1	82	737	1620	4.553	1132	1118
173	(E)-Rose oxide	876-18-6	139	680	1626	3.149	1133	1127
174	Ethyl 3-hydroxyhexanoate	2305-25-1	71	786	1626	3.617	1134	1133
175	p-Menth-3-en-1-ol	586-82-3	81	691	1650	3.349	1143	1138
176	N-Isopentylacetamide	13434-12-3	72	882	1668	4.786	1149	1150
177	o-Dimethoxybenzene	91-16-7	138	818	1674	5.389	1151	1154
178	Isobutyl hexanoate	105-79-3	99	907	1680	2.798	1152	1144
179	4-Oxoisophorone	1125-21-9	68	839	1680	4.994	1153	1142
180	Prehnitene	488-23-3	119	905	1686	3.753	1155	1120
181	Camphor	464-49-3	95	762	1686	4.207	1155	1151
182	Nerol oxide	1786-08-9	83	820	1692	3.462	1156	1151
183	Pentylbenzene	538-68-1	91	783	1704	3.214	1161	1154
184	(Z)-3-Nonenol	10340-23-5	81	812	1704	3.237	1161	1160
185	γ-Heptalactone	105-21-5	85	802	1704	5.818	1162	1144
186	Menthone	89-80-5	112	756	1710	3.577	1162	1154
187	2-Methylundecane	7045-71-8	85	847	1716	1.936	1165	1165
188	3-Cyclohexene-1-carboxaldehyde, 1,3,4-trimethyl-	40702-26-9	137	752	1722	3.571	1167	1171
189	3-Ethylphenol	620-17-7	107	710	1722	4.408	1168	1184
190	Benzyl acetate	140-11-4	150	880	1728	4.877	1170	1165
191	3-Methylundecane	1002-43-3	57	849	1734	1.968	1171	1169
192	(Z)-6-Nonenol	35854-86-5	67	872	1734	3.206	1171	1172
193	Isomenthone	491-07-6	112	814	1734	3.787	1171	1165
194	m-Dimethoxybenzene	151-10-0	138	864	1740	5.095	1174	1182
195	Ocimenol	5986-38-9	93	738	1746	3.309	1175	1179
196	Ethyl benzoate	93-89-0	105	906	1746	4.527	1177	1180
197	Isobutyl methoxypyrazine	24683-00-9	124	618	1758	3.703	1180	1179
198	m-Methylacetophenone	585-74-0	119	760	1758	5.071	1180	1183
199	1-Nonanol	143-08-8	70	907	1764	2.995	1182	1173
200	(E)-Linalool oxide	14049-11-7	59	797	1764	3.755	1181	1184
201	Phenethyl formate	104-62-1	104	890	1764	4.901	1183	1178
202	Methyl benzeneacetate	101-41-7	150	838	1764	5.175	1183	1194
203	Diethyl succinate	123-25-1	74	890	1770	4.325	1184	1191
204	4-Ethyl phenol	123-07-9	107	930	1776	4.682	1186	1178
205	Terpinen-4-ol	562-74-3	71	859	1782	3.532	1189	1177
206	1-Dodecene	112-41-4	69	903	1794	2.165	1192	1193
207	Octanoic acid	124-07-2	144	844	1800	3.435	1194	1202
208	Dill ether	74410-10-9	137	751	1800	3.861	1193	1184
209	Naphthalene	91-20-3	128	855	1800	5.179	1194	1191
210	p-Methylacetophenone	122-00-9	119	793	1806	5.064	1196	1179
211	Dodecane[d]	112-40-3	57	852	1818	2.227	1201	1200
212	Methyl salicylate	119-36-8	120	913	1824	4.894	1202	1201
213	p-Creosol	93-51-6	123	862	1836	4.863	1206	1188
214	α-Terpineol	98-55-5	136	850	1842	3.603	1210	1186
215	Safranal	116-26-7	150	799	1848	4.385	1211	1196
216	Decanal	112-31-2	82	869	1854	3.083	1213	1206
217	Benzofuran, 4,7-dimethyl-	28715-26-6	145	828	1860	4.364	1217	1220
218	4,7-Dimethylbenzofuran	28715-26-6	145	829	1878	4.378	1223	1220
219	Methyl nonanoate[e]	1731-84-6	141	892	1890	3.003	1226	1229
220	Ethyl nicotinate	614-18-6	106	812	1890	5.045	1226	1218
221	p-Menth-1-en-9-al	29548-14-9	94	764	1896	3.993	1228	1217
222	β-Cyclocitral	432-25-7	137	874	1896	4.196	1229	1220
223	Citronellol	106-22-9	156	899	1908	3.288	1233	1233
224	2-Hydroxycineol	18679-48-6	108	756	1914	4.201	1236	1227
225	Benzothiazole	95-16-9	135	911	1926	0.497	1239	1244
226	6-Ethyl-o-cresol	1687-64-5	121	859	1926	4.499	1239	1236
227	Benzenepropanol	122-97-4	117	851	1926	5.121	1241	1231
228	Isothiocyanatocyclohexane	1122-82-3	141	860	1932	4.925	1243	1260

Table 3 (*Continued*)

Peak#	Compound	CAS	Unique mass[a]	MS match	1 RT(s)	2 RT(s)	RI[b] (calc)	RI[c] (lit)
229	Ethyl phenylacetate	101-97-3	164	908	1950	4.857	1249	1247
230	Ethyl 2-octenoate	2351-90-8	125	862	1956	3.309	1250	1243
231	2-Methylbutyl hexanoate	2601-13-0	99	874	1962	2.875	1252	1247
232	Isopentyl hexanoate	2198-61-0	99	898	1962	2.875	1252	1250
233	D-Carvone	2244-16-8	82	767	1962	4.509	1253	1254
234	2-Nitro-p-cresol	119-33-5	153	781	1968	5.031	1255	1250
235	Geraniol	106-24-1	69	818	1974	3.596	1257	1255
236	Carvotanacetone	499-71-8	82	764	1974	4.286	1258	1246
237	α-Ionene	475-03-6	159	629	1986	3.320	1261	1256
238	2-Phenylethyl acetate	103-45-7	91	906	1986	4.877	1262	1256
239	γ-Octalactone	104-50-7	85	850	1992	5.575	1264	1262
240	9-Decenol	13019-22-2	68	802	2010	3.258	1270	1267
241	3,5-Dimethoxytoluene	4179-19-5	152	842	2016	4.895	1273	1276
242	Nonanoic acid	112-05-0	60	696	2028	2.336	1277	1280
243	1-Decanol	112-30-1	70	921	2028	3.067	1277	1283
244	Ethyl salicylate	118-61-6	120	858	2028	4.511	1277	1267
245	4-Ethylguaiacol	2785-89-9	137	926	2040	4.755	1281	1282
246	Diethyl glutarate	818-38-2	143	915	2046	4.164	1283	1284
247	Vitispirane	65416-59-3	192	904	2058	3.493	1287	1272
248	Phellandral	21391-98-0	109	814	2058	4.303	1287	1273
249	δ-Octalactone	698-76-0	99	866	2070	0.069	1291	1287
250	p-Ethylacetophenone	937-30-4	133	689	2070	4.963	1292	1281
251	Propyl octanoate	624-13-5	145	895	2076	2.919	1294	1290
252	2-Undecanone	112-12-9	58	885	2082	3.143	1296	1295
253	(E)-Oak lactone	39638-67-0	99	827	2082	5.011	1297	1304
254	Ethyl nonanoate	123-29-5	88	895	2088	2.931	1298	1295
255	Perilla alcohol	536-59-4	68	760	2088	4.222	1299	1295
256	Thymol	89-83-8	135	831	2088	4.332	1298	1290
257	Tridecane[d]	629-50-5	57	849	2094	2.083	1300	1300
258	p-Cymen-7-ol	536-60-7	135	850	2094	4.722	1301	1295
259	Theaspirane A	0-00-0	138	844	2106	3.283	1305	1301
260	2-Undecanol	1653-30-1	45	886	2112	2.831	1306	1303
261	p-Menth-1-en-9-ol	18479-68-0	94	797	2112	4.021	1308	1295
262	Carvacrol	499-75-2	135	855	2112	4.433	1307	1304
263	Edulan I	41678-29-9	177	768	2136	3.705	1317	1309
264	4-Hydroxy-3-methylacetophenone	876-02-8	135	839	2136	5.715	1317	1323
265	4-Vinylguaiacol	7786-61-0	150	825	2142	5.287	1319	1317
266	Theaspirane B	0-00-0	138	822	2148	3.395	1322	1319
267	Methyl decanoate	110-42-9	74	873	2160	3.004	1325	1323
268	Methyl geranate	2349-14-6	114	868	2160	3.596	1325	1326
269	(Z)-Oak lactone	55013-32-6	71	920	2166	5.350	1329	1340
270	Isobutyl octanoate	5461-06-3	127	856	2220	2.811	1348	1348
271	Citronellol acetate	150-84-5	81	752	2226	3.191	1350	1352
272	Ethyl dihydrocinnamate	2021-28-5	104	858	2232	4.632	1354	1350
273	Syringol	91-10-1	154	859	2244	0.360	1356	1362
274	Eugenol	97-53-0	164	915	2250	4.933	1360	1359
275	TDN	30364-38-6	157	807	2256	4.137	1361	1364
276	(Z)-β-Damascenone	23696-85-7	121	786	2262	4.101	1364	1367
277	γ-Nonalactone	104-61-0	85	883	2268	5.315	1368	1361
278	Dihydroeugenol	2785-87-7	137	924	2274	4.600	1369	1365
279	Hydroxy citronellol	107-74-4	59	793	2286	2.817	1373	1359
280	1-Undecanol	112-42-5	126	855	2298	3.032	1378	1367
281	(E)-α-Ionol	25312-34-9	138	770	2304	3.464	1381	1376
282	(E)-β-Damascenone	23726-93-4	121	886	2316	4.263	1385	1387
283	Biphenyl	92-52-4	154	894	2322	5.345	1388	1385
284	Ethyl decanoate	110-38-3	101	620	2325	3.225	1388	1393
285	Methyl cinnamate	103-26-4	131	796	2334	5.381	1393	1397
286	2-Phenylethyl isobutyrate	103-48-0	104	771	2346	4.419	1397	1396
287	Tetradecane[d]	629-59-4	57	869	2358	2.129	1401	1400
288	α-Cedrene	469-61-4	119	685	2391	3.762	1414	1410
289	β-Damascone	85949-43-5	177	760	2394	4.098	1415	1419
290	Dihydro-α-ionone	31499-72-6	136	699	2406	3.819	1420	1406
291	α-Ionone	127-41-3	136	687	2424	3.931	1428	1426
292	1,7-Dimethylnaphthalene	575-37-1	156	896	2436	5.087	1433	1419
293	Aromadendrene	109119-91-7	161	809	2454	3.077	1439	1443
294	2-Phenylethyl butyrate	103-52-6	104	858	2466	4.506	1445	1439
295	Isoamyl octanoate	2035-99-6	127	859	2472	2.880	1447	1450
296	Dihydropseudoionone	689-67-8	69	838	2481	3.658	1451	1457
297	β-Farnesene	18794-84-8	93	854	2490	2.906	1454	1455
298	DBQ	719-22-2	220	833	2520	3.741	1467	1472
299	γ-Decalactone	706-14-9	85	792	2532	5.134	1472	1470
300	1-Dodecanol	112-53-8	97	874	2544	3.055	1477	1483
301	Cabreuva oxide D	107602-52-8	94	868	2556	3.403	1481	1479
302	dehydro-β-Ionone	1203-08-3	175	914	2556	4.447	1483	1485
303	δ-Decenolactone	54814-64-1	97	841	2556	5.710	1482	1483
304	α-Curcumene	644-30-4	132	795	2562	3.415	1484	1485
305	β-Ionone	79-77-6	177	828	2562	4.174	1485	1486

Table 3 (*Continued*)

Peak#	Compound	CAS	Unique mass[a]	MS match	1 RT(s)	2 RT(s)	RI[b] (calc)	RI[c] (lit)
306	Propyl decanoate	30673-60-0	61	852	2580	2.911	1491	1489
307	Ethyl undecanoate	627-90-7	88	879	2586	2.922	1494	1491
308	(Z)-β-Guaiene	88-84-6	161	737	2586	3.393	1493	1492
309	1,10-Oxidocalamenene	143785-42-6	173	925	2586	4.228	1494	1491
310	Isoamyl phenylacetate	102-19-2	70	844	2586	4.400	1494	1490
311	Phenethyl isovalerate	140-26-1	104	831	2592	4.269	1496	1490
312	δ-Decalactone	705-86-2	99	831	2598	5.550	1500	1505
313	Pentadecane[d]	629-62-9	57	884	2604	2.159	1499	1500
314	α-Amorphene	483-75-0	105	882	2610	3.335	1504	1505
315	α-Farnesene	502-61-4	189	607	2616	3.755	1506	1511
316	Butylated hydroxytoluene	128-37-0	205	873	2616	3.806	1506	1533
317	2,4-Di-tert-butylphenol	96-76-4	191	863	2622	3.938	1510	1513
318	β-Bisabolene	495-61-4	204	783	2628	3.087	1512	1509
319	α-Alaskene	28400-12-6	136	632	2628	3.886	1511	1512
320	Methyl dodecanoate	111-82-0	74	846	2658	2.997	1524	1525
321	δ-Cadinene	483-76-1	134	737	2658	3.444	1524	1528
322	α-Panasinsen	56633-28-4	161	610	2658	3.450	1524	1518
323	(E)-Calamene	483-77-2	159	781	2670	3.787	1529	1530
324	Ethyl 4-ethoxybenzoate	23676-09-7	121	827	2670	4.969	1530	1522
325	β-Sesquiphellandrene	20307-83-9	93	668	2676	3.259	1532	1526
326	Isolongifolene, 4,5,9,10-dehydro-	156747-45-4	200	780	2682	4.192	1535	1544
327	Ethyl 3-hydroxytridecanoate	107141-15-1	117	824	2688	3.492	1537	1539
328	Dihydroactinidiolide	17092-92-1	111	860	2706	0.410	1543	1548
329	Isobutyl decanoate	30673-38-2	155	881	2706	2.814	1546	1545
330	α-Calacorene	21391-99-1	157	926	2718	4.085	1550	1549
331	Nerolidol	7212-44-4	93	814	2748	3.343	1563	1566
332	β-Calacorene	50277-34-4	157	862	2766	4.189	1572	1564
333	β-Vetivenene	27840-40-0	187	882	2772	4.728	1575	1554
334	γ-Undecalactone	104-67-6	85	702	2784	4.977	1580	1573
335	Hexyl octanoate	1117-55-1	127	816	2790	2.920	1583	1584
336	Ethyl dodecanoate	106-33-2	101	865	2820	2.965	1595	1593
337	Hexadecane[d]	544-76-3	57	887	2832	2.194	1600	1600
338	Isopropyl laurate	10233-13-3	60	851	2892	2.759	1627	1618
339	Cubenol	21284-22-0	161	762	2928	4.001	1643	1642
340	Isopentyl decanoate	2306-91-4	70	885	2934	2.863	1646	1647
341	Phenethyl hexanoate	6290-37-5	104	846	2934	4.363	1648	1650
342	Cadalene	483-78-3	183	886	3018	4.763	1684	1684
343	α-Bisabolo	515-69-5	119	893	3036	3.767	1694	1688
344	Ethyl tridecanoate	28267-29-0	88	845	3042	2.915	1695	1687
345	Heptadecane[d]	629-78-7	57	869	3054	2.222	1700	1700
346	Methyl tetradecanoate	124-10-7	74	720	3108	2.992	1726	1722
347	2,6-Diisopropylnaphthalene	24157-81-1	197	865	3120	4.307	1732	1728
348	(Z)-Farnesol	3790-71-4	69	776	3132	3.173	1737	1718
349	Ethyl 3-hydroxydodecanoate	126679-28-5	117	736	3144	3.412	1743	1743
350	Ethyl tetradecanoate	124-06-1	88	866	3252	2.923	1795	1796
351	Octadecane[d]	593-45-3	57	864	3264	2.249	1800	1800
352	Isopropyl myristate	110-27-0	102	791	3312	2.777	1825	1823
353	Isoamyl laurate	6309-51-9	70	826	3354	2.857	1846	1847
354	Phenethyl octanoate	5457-70-5	104	860	3372	4.198	1856	1846
355	Ethyl pentadecanoate	41114-00-5	88	884	3450	2.920	1897	1897
356	Dibutyl phthalate	84-74-2	149	908	3582	5.233	1965	1967
357	Ethyl 9-hexadecenoate	54546-22-4	79	808	3606	3.135	1976	1977
358	Ethyl hexadecanoate	628-97-7	88	889	3642	2.932	1995	1994
359	Eicosane[d]	112-95-8	57	867	3654	2.300	2000	2000
360	Isopropyl palmitate	142-91-6	102	710	3696	2.778	2022	2027
361	Ethyl octadecanoate	111-61-5	88	741	4008	2.912	2182	2194
T1	Mercaptoacetone	24653-75-6	90	898	438	2.342	726	
T2	2-(Methoxymethyl)furan	13679-46-4	81	861	720	3.204	829	
T3	Ethyl 3-furoate	614-98-2	95	864	1224	3.957	1000	
T4	Pantolactone	599-04-2	71	874	1404	5.508	1060	
T5	2-Thiopheneacetic acid	1918-77-0	97	758	1410	4.300	1061	
T6	Ethyl levulate	539-88-8	99	777	1422	4.829	1066	
T7	γ-Ethoxybutyrolactone	932-85-4	85	914	1428	5.955	1069	
T8	Isoamyl lactate	19329-89-6	45	843	1440	3.210	1071	
T9	Ethyl methyl succinate	627-73-6	115	903	1554	4.477	1109	
T10	(E)-2-Ethyl heptenoate	54340-72-6	111	758	1680	3.305	1152	
T11	(E)-6-Nonenol	31502-19-9	67	804	1764	3.296	1181	
T12	Ethyl 2-pyrrolecarboxylate	2199-43-1	139	801	1836	5.510	1207	
T13	Diethyl methylsuccinate	4676-51-1	143	799	1842	3.913	1209	
T14	p-tert-Butylcyclohexanone	98-53-3	98	809	1920	4.216	1237	
T15	3,9-Epoxy-p-menth-1-ene	70786-44-6	137	774	1932	4.115	1241	
T16	Diethyl malate	626-11-9	117	880	2010	4.667	1270	
T17	Ethyl 5-oxotetrahydro-2-furancarboxylate	1126-51-8	85	930	2112	1.342	1307	
T18	2-Hexanoylfuran	14360-50-0	110	820	2112	4.470	1309	
T19	Isoamyl 2-furoate	615-12-3	95	871	2136	4.389	1317	

Table 3 (*Continued*)

Peak#	Compound	CAS	Unique mass[a]	MS match	1 RT(s)	2 RT(s)	RI[b] (calc)	RI[c] (lit)
T20	3,4-Dihydro-3-oxoedulan	20194-67-6	193	849	2568	4.549	1487	
T21	Megastigmatrienone	38818-55-2	148	782	2796	4.829	1587	
T22	Heptyl ketone	818-23-5	57	870	2994	2.976	1674	

Note: RI (calc) values for compounds 1–21 are extrapolated using ChromaTOF Software and RI (lit) values could not be found for compounds T1–T22 therefore identification is based on MS match only.

[a] Unique ion (*m*/*z*): used for peak area determination, identified as the unique ion by ChromaTOF data analysis.

[b] Retention indices calculated from C_8 to C_{20} n-alkanes.

[c] Retention indices reported in the literature for 5% phenyl polysilphenylene-siloxane capillary GC columns or equivalents [33,34].

[d] Straight chain n-alkanes not present in the wine samples.

[e] Methyl nonanoate internal standard not present in wine samples.

by the Metabolomics Standards Initiative (MSI) Chemical Analysis Working Group (CAWG) [49]. In the analysis of complex biological samples both MS and RI information are prerequisite for unambiguous compound identification [49].

Data analysis using ChromaTOF identified a total of 375 compounds, plus the 7 alkanes and the 1 internal standard, which had an average mass spectral match of 838 with an upper and lower 95% of the mean at 844 and 831, respectively. The calculated retention index values were also compared to van den Dool and Kratz retention indices [50] reported in the literature with an average difference in the RI values of 5.4 units with an upper and lower 95% of the mean at 6.0 and 4.7, respectively. Bianchi et al. commented that differences in retention indices for aroma compounds on comparable stationary phases may vary between 5 and 20 units, however, larger differences have been observed [51]. Babushok et al. also noted that in the development of the NIST database of retention indices, 80,427 retention indices representing 9722 species analyzed on dimethylpolysiloxane stationary phases had an average deviation of 10 units but a 99th percentile deviation of 91 units [52]. The differences in calculated and reported retention indices reported in this study fall well within these values. Compounds where retention indices have not been reported in the literature have been listed at the end of Table 3 while compounds that were not in good agreement with both mass spectral match and literature RI values were not included.

The majority of current non-targeted GC–MS methodologies tentatively identify ~30–60 analytes in a single analysis [53–55] with many other methods developed for targeted and quantitative analysis of fewer but more specific compounds [56–59]. A recent three paper series [16,19,60] tentatively identify a total of 201 wine aroma compounds from Ice-wine using a high throughput HS-SPME GC-TOFMS method. However, on review of the data presented in Table 2 of the second paper [16] tentative identifications included 118 analytes that were not compared to literature retention indices (RI), 26 analytes were >40 RI units different to reported literature RIs, 11 analytes were classified as Unknowns, 71 analytes were quantified using masses that were <10% of the base peak, and 6 analytes were quantified using masses larger than the molecular weight of the assigned analyte. This subsequently reduced the total number of tentatively identified analytes from 201 to a subset of 30 where the calculated RI was within 40 RI units of a literature RI value and where the reported quantification mass was >10% of the base peak. This figure is more in-line with that reported in other single dimensional GC–MS methodologies.

This suggests that most current analytical methods are capable of identifying at most ~10% of the known volatile compounds reported in wines. The current study has demonstrated an optimized analytical method capable of analyzing volatile compounds in wine with a number of compounds tentatively identified at an

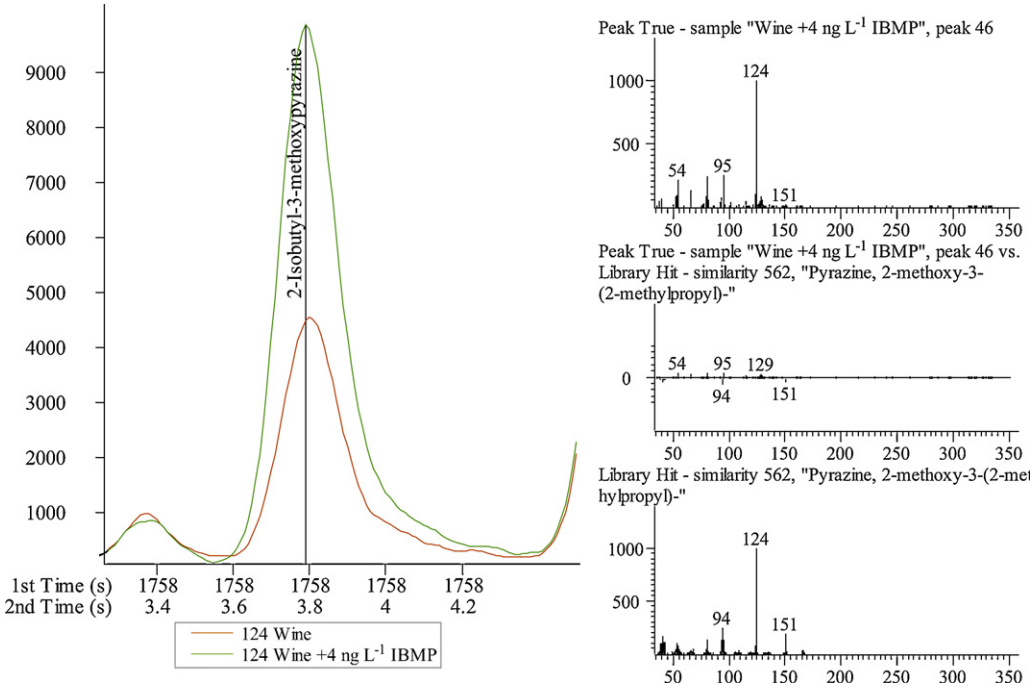

Fig. 8. Identifies the deconvoluted peak for IBMP in a wine and the same wine spiked with ~4 ng L^{-1} of the same compound. Note the deconvoluted Peak True mass spectrum provides additional confirmation on the quality of the spectral match.

order of magnitude greater than most current single dimensional GC–MS methodologies.

3.6. Differentiating commercial wines using volatile profiling

The volatiles in commercial Cabernet Sauvignon wines, from different producers, growing regions and vintages, were run in triplicate and analyzed using a one-way analysis of variance for each compound identified in Table 3. Of the 375 compounds identified in the commercial wines, 324 compounds were significantly different between the wines to a significance of 0.05 using a Tukey–Kramer HSD test (data not presented). Given that the commercial products were from different producers, growing regions and vintages it is not unexpected that there would be differences among the products. The results of this method evaluation clearly demonstrate that the method developed has the capacity to resolve and identify a large number of compounds and could be used to differentiate wines based on their volatile profile which will be the subject of further work.

4. Conclusions

The current study has described the development of a sensitive and comprehensive method for analyzing volatile and semi-volatile compounds found in the wine headspace through the use of HS-SPME/GC × GC-TOFMS. This study is the first to clearly show that the use of elevated temperatures during the incubation step of HS-SPME analysis of wine does generate artifacts. It is not intended that this method be used for high throughput or routine analysis of wine volatiles due to the higher costs currently associated with the cryogenic modulation required for GC × GC analysis of low molecular weight volatile compounds. However, further development of consumable-free modulation may extend the application of this analytical technology to production areas of the wine industry for quality assurance and quality control. It is intended that in the immediate future, wine aroma research and wine sensory research will utilize this non-targeted method to assess compositional changes in the wine volatile profile.

Acknowledgements

This research project was funded by Australia's grape growers and winemakers through their investment body the Grape and Wine Research and Development Corporation with matching funding from the Australian Federal Government. This work has been conducted as a collaboration between Murdoch University, CSIRO Plant Industry and The University of California, Davis with industry support provided by Houghton Wines. The authors would like to acknowledge that the GC × GC TOFMS was purchased through an Australian Research Council (ARC) Large Equipment Infrastructure and Facilities (LEIF) grant. The authors would also like to thank Dr. Pete Stevens, Mr. Lucas Smith and Mr. Berthold Franz (LECO, St. Joseph, MI, USA), and Dr. Roger Boulton (University of California, Davis, CA, USA) for their helpful discussions and suggestions.

References

[1] V. Ferreira, R. López, J.F. Cacho, J. Sci. Food Agric. 80 (2000) 1659.
[2] I.L. Francis, J.L. Newton, J. Aust, Grape Wine Res. 11 (2005) 114.
[3] A. Rapp, Fresen. J. Anal. Chem. 337 (1990) 777.
[4] A. Escudero, E. Campo, L. Fariña, J. Cacho, V. Ferreira, J. Agric. Food Chem. 55 (2007) 4501.
[5] W.S. Cain, M. Drexler, Ann. N. Y. Acad. Sci. 237 (1974) 427.
[6] T. Miyazawa, M. Gallagher, G. Preti, P.M. Wise, Chem. Senses 33 (2008) 363.
[7] B. Pineau, J.C. Barbe, C. Van Leeuwen, D. Dubourdieu, J. Agric. Food Chem. 55 (2007) 4103.
[8] D. Ryan, P.D. Prenzler, A.J. Saliba, G.R. Scollary, Trends Food Sci. Technol. 19 (2008) 383.
[9] Z. Liu, J.B. Phillips, J. Chromatogr. Sci. 29 (1991) 227.
[10] R.C.Y. Ong, P.J. Marriott, J. Chromatogr. Sci. 40 (2002) 276.
[11] J. Dallüge, J. Beens, U.A.Th. Brinkman, J. Chromatogr. A 1000 (2003) 69.
[12] T. Górecki, J. Harynuk, O. Panic, J. Sep. Sci. 27 (2004) 359.
[13] M. Adahchour, J. Beens, U.A.Th. Brinkman, J. Chromatogr. A 1186 (2008) 67.
[14] E. Marengo, M. Aceto, V. Maurino, J. Chromatogr. A 943 (2001) 123.
[15] J.S. Câmara, M.A. Alves, J.C. Marques, Food Chem. 101 (2007) 475.
[16] L. Setkova, S. Risticevic, J. Pawliszyn, J. Chromatogr. A 1147 (2007) 224.
[17] K.L. Howard, J.H. Mike, R. Riesen, Am. J. Enol. Viticult. 56 (2005) 37.
[18] J.S. Câmara, M.A. Alves, J.C. Marques, Anal. Chim. Acta 555 (2006) 191.
[19] L. Setkova, S. Risticevic, J. Pawliszyn, J. Chromatogr. A 1147 (2007) 213.
[20] D. Ryan, P. Watkins, J. Smith, M. Allen, P. Marriott, J. Sep. Sci. 28 (2005) 1075.
[21] S.M. Rocha, E. Coelho, J. Zrostlíková, I. Delgadillo, M.A. Coimbra, J. Chromatogr. A 1161 (2007) 292.
[22] I. Ryona, B.S. Pan, D.S. Intrigliolo, A.N. Lakso, G.L. Sacks, J. Agric. Food Chem. 56 (2008) 10838.
[23] I. Ryona, B.S. Pan, G.L. Sacks, J. Agric. Food Chem. 57 (2009) 8250.
[24] I. Ryona, S. Leclerc, G.L. Sacks, J. Agric. Food Chem. 58 (2010) 9723.
[25] H.G. Schmarr, J. Bernhardt, U. Fischer, A. Stephan, P. Müller, D. Durner, Anal. Chim. Acta 672 (2010) 114.
[26] R. Perestrelo, S. Petronilho, J.S. Câmara, S.M. Rocha, J. Chromatogr. A 1217 (2010) 3441.
[27] A. Cox, D.L. Capone, G.M. Elsey, M.V. Perkins, M.A. Sefton, J. Agric. Food Chem. 53 (2005) 3584.
[28] C. Wood, T.E. Siebert, M. Parker, D.L. Capone, G.M. Elsey, A.P. Pollnitz, M. Eggers, M. Meier, T. Vossing, S. Widder, J. Agric. Food Chem. 56 (2008) 3738.
[29] P. Polášková, J. Herszage, S.E. Ebeler, Chem. Soc. Rev. 37 (2008) 2478.
[30] D. Roujou de Boubée, C. Van Leeuwen, D. Dubourdieu, J. Agric. Food Chem. 48 (2000) 4830.
[31] Y. Wang, J. O'Reilly, Y. Chen, J. Pawliszyn, J. Chromatogr. A 1072 (2005) 13.
[32] L. Setkova, S. Risticevic, C.M. Linton, G. Ouyang, L.M. Bragg, J. Pawliszyn, Anal. Chim. Acta 581 (2007) 221.
[33] R.P. Adams, Identification of Essential Oil Components by Gas Chromatography/Mass Spectorscopy, 4th ed., Allured Publishing Corporation, Carol Stream, IL, 2007.
[34] S.E. Stein, in: P.J. Linstrom, W.G. Mallard (Eds.), NIST Chemistry WebBook, NIST Standard Reference Database Number 69, National Institute of Standards and Technology, Gaithersburg, MD, 2009.
[35] J.H. Ward Jr., J. Am. Stat. Assoc. 58 (1963) 236.
[36] R.M. Mazo, J. Phys. Chem. B 110 (2006) 24077.
[37] N. Ni, M.M. El-Sayed, T. Sanghvi, S.H. Yalkowsky, J. Pharm. Sci. 89 (2000) 1620.
[38] N. Ni, S.H. Yalkowsky, Int. J. Pharm. 254 (2003) 167.
[39] V. Ferreira, M. Ardanuy, R. López, J.F. Cacho, J. Agric. Food Chem. 46 (1998) 4341.
[40] T. Čajka, J. Hajšlová, J. Cochran, K. Holadová, E. Klimánková, J. Sep. Sci. 30 (2007) 534.
[41] A.C. Silva Ferreira, P. Guedes De Pinho, P. Rodrigues, T. Hogg, J. Agric. Food Chem. 50 (2002) 5919.
[42] A.C. Silva Ferreira, P. Guedes De Pinho, Anal. Chim. Acta 513 (2004) 169.
[43] P. Winterhalter, M.A. Sefton, P.J. Williams, Am. J. Enol. Viticult. 41 (1990) 277.
[44] G. Versini, S. Carlin, A. Dalla Serra, G. Nicolini, A. Rapp, in: P. Winterhalter, R. Rouseff (Eds.), ACS Symp. Ser., American Chemical Society, Washington, DC, 2002, p. 285.
[45] J.M. Davis, D.R. Stoll, P.W. Carr, Anal. Chem. 80 (2008) 461.
[46] J. Dallüge, R.J.J. Vreuls, J. Beens, U.A.Th. Brinkman, J. Sep. Sci. 25 (2002) 201.
[47] R.E. Murphy, M.R. Schure, J.P. Foley, Anal. Chem. 70 (1998) 1585.
[48] J.J. Harynuk, A.H. Kwong, P.J. Marriott, J. Chromatogr. A 1200 (2008) 17.
[49] L. Sumner, A. Amberg, D. Barrett, M. Beale, R. Beger, C. Daykin, T. Fan, O. Fiehn, R. Goodacre, J. Griffin, T. Hankemeier, N. Hardy, J. Harnly, R. Higashi, J. Kopka, A. Lane, J. Lindon, P. Marriott, A. Nicholls, M. Reily, J. Thaden, M. Viant, Metabolomics 3 (2007) 211.
[50] H. van den Dool, P.D. Kratz, J. Chromatogr. 11 (1963) 463.
[51] F. Bianchi, M. Careri, A. Mangia, M. Musci, J. Sep. Sci. 30 (2007) 563.
[52] V.I. Babushok, P.J. Linstrom, J.J. Reed, I.G. Zenkevich, R.L. Brown, W.G. Mallard, S.E. Stein, J. Chromatogr. A 1157 (2007) 414.
[53] A. Tredoux, A. de Villiers, P. Májek, F. Lynen, A. Crouch, P. Sandra, J. Agric. Food Chem. 56 (2008) 4286.
[54] R. Perestrelo, M. Caldeira, F. Rodrigues, J.S. Câmara, J. Sep. Sci. 31 (2008) 1841.
[55] J. Gallardo-Chacón, S. Vichi, E. López-Tamames, S. Buxaderas, J. Agric. Food Chem. 57 (2009) 3279.
[56] T.E. Siebert, H.E. Smyth, D.L. Capone, C. Neuwöhner, K.H. Pardon, G.K. Skouroumounis, M.J. Herderich, M.A. Sefton, A.P. Pollnitz, Anal. Bioanal. Chem. 381 (2005) 937.
[57] Y.S. Kotseridis, M. Spink, I.D. Brindle, A.J. Blake, M. Sears, X. Chen, G. Soleas, D. Inglis, G.J. Pickering, J. Chromatogr. A 1190 (2008) 294.
[58] L. Mateo-Vivaracho, J. Cacho, V. Ferreira, J. Chromatogr. A 1185 (2008) 9.
[59] V. Canuti, M. Conversano, M.L. Calzi, H. Heymann, M.A. Matthews, S.E. Ebeler, J. Chromatogr. A 1216 (2009) 3012.
[60] J.L. Giraudel, L. Setkova, J. Pawliszyn, M. Montury, J. Chromatogr. A 1147 (2007) 241.