

Dehydration of 2-methylisoborneol to 2-methyl-2-bornene in the Trace Analysis of Taste-Odorants in Water by Purge-and-Trap Sampling with Gas Chromatography (GC) -Mass Selective (MS) Detection

Thavrin Manickum* and Wilson John

Scientific Services Laboratories, Engineering & Scientific Services Division, Head Office, Level 3, Umgeni Water, 310 Burger Street, Pietermaritzburg 3201, KwaZulu-Natal, South Africa

Abstract

A "salt-free", automated purge-and-trap sampling method, with gas chromatographic-mass spectrometric detection for simultaneous detection of 2-methylisoborneol (2-MIB) and geosmin (GSM) was developed. The preliminary procedure involved purging a 25 mL water sample, at 80°C for 11 min, with helium gas, and trapping onto a Tenax sorbent. Target analytes were desorbed with helium gas onto the gas chromatograph, coupled to a mass spectrometer, via a transfer line. Chromatographic separation was performed on HP5-MS capillary column (30 m x 0.25 mm x 0.25 µm), followed by mass spectrometric detection in the selected ion monitoring mode. During the preliminary method optimization and development stages, inconsistencies were noted regarding the relative ratios of ions m/z 95 and 107, two ion fragments frequently used for identification and subsequent quantitation of 2-MIB. NIST reference mass spectra, liquid auto sampler injection of commercial standards of the target analytes, and gas chromatographic retention times were used to confirm peak identification on the chromatograms of aqueous standards analyzed by purge-and-trap, with mass selective detection. The effect of mass spectrometer source temperature and use of a 6 mm drawout plate in the source body, on the relative ratios of m/z 95, 107, 108, for 2-MIB, and m/z 111, 112, 125, for GSM, and precision, was investigated. It was found that use of the latter had no significant effect on the ratios. The observed data indicated that 2-MIB dehydrates to 2-methyl-2-bornene (2-M-2-B) during the purge-and-trap cycle conditions used, presumably when the sample is initially purged at 80°C. The relative retention times were ± 9.2min, ± 12.0min and ± 15.2min, for 2-M-2-B, 2-MIB and GSM, respectively, under the gas chromatographic conditions. Preliminary results indicate suitability of the technique for trace analysis of GSM in water samples over the 5-100 ng/L analytical range.

Keywords: Purge-and-trap; Mass spectrometer; Ion source; Geosmin; 2-Methylisoborneol; Gas chromatography-mass spectrometry; 2-Methyl-2-bornene

Introduction

Geosmin (GSM) and 2-methylisoborneol (2-MIB), semi-volatile compounds produced by a wide range of aquatic and blue-green algae species, contribute to the earthy-musty taste and odor problems of water supplies [1].

We recently reported a "salt-free" purge-and-trap method, on the Tekmar-Stratum equipment, using gas chromatography (GC), with mass selective (MS) detection [2] for trace analysis of these taste-odorants in water matrices.

Various detection systems have been reported for analysis of these compounds, like flame ionization [3], atomic emission [4], electron-capture [5] and olfactometry [6]. Gas chromatography [7,8], in combination with mass spectrometric detection [9] offers excellent sensitivity and selectivity.

Of the various sample concentration techniques for extraction of 2-MIB and geosmin from water samples, purge-and-trap, coupled with mass spectrometric detection [2,3,10-12], is a fairly rapid sample concentration-extraction technique.

Mass spectrometry [13-16] is a common detection system due to its obvious, powerful usefulness as an analytical confirmatory technique.

For mass spectrometric detection methods, the common mass fragments used for analysis of 2-MIB, and GSM, in the selected ion monitoring (SIM) mode is m/z 95, 107 and 112, respectively, as shown

in Table 1. A review of the literature to date indicates no data or comment on the relative ratios of mass fragment ions 95 and 107 for 2-MIB in purge-trap sample concentration techniques, using GC, with MS as a detection system.

In our method optimization and development using the recently launched Eclipse purge-and-trap sample concentrator, from OI Analytical, we noted erratic relative ratios for ions m/z 95 and 107 for 2-MIB, common mass fragments used for identification and quantification of 2-MIB. Unnecessary changes in these ratios will obviously contribute negatively to observed signal responses, commonly peak area, or peak height, and hence affect precision, accuracy, and subsequent quantitation of the target analytes.

We here with report our novel findings, regarding the relative ratios of m/z 95 and 107, two mass fragments frequently used in the

*Corresponding author: Thavrin Manickum, Scientific Services Laboratories, Engineering & Scientific Services Division, Head Office, Level 3, Umgeni Water, 310 Burger Street, Pietermaritzburg 3201, KwaZulu-Natal, South Africa, Tel: +27 33 341 1067; Fax: +27 33 341 1501; E-mail: thavrin.manickum@umgeni.co.za

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analysis of 2-MIB, in purge-and-trap/GCMS, which has not to date been investigated or reported.

Experimental

Chemicals and consumables

(±)-Geosmin (2 mg/mL) and 2-MIB (10 mg/mL) in methanol, of greater than 98% purity, were obtained from Sigma (St. Louis, USA) [28].

HPLC grade methanol was obtained from Merck Darmstadt. Ultrahigh purity helium gas (99.999%) for GC-MS was obtained from Air Products, Durban, South Africa. Water was obtained from a Milli-Q (MQ) (MILLIPORE) water purification system (Millipore, USA). The conductivity was on average 0.054 $\mu\text{S}/\text{cm}$ (range: 0.048-0.060 $\mu\text{S}/\text{cm}$). The # 7 Tenax trap, Part # 227348, was supplied by OI Analytical on commissioning of the equipment.

The purge-trap “narrow bore” liner (Part number: 18740-80220), for purge-and trap sampling, was obtained from Chemetrix Ltd, Authorised Distributor, Agilent Technologies LCSA Products, Halfway House, South African agents for Agilent; the Supelco split liner (Part number: 2-0510,05), for liquid auto sampler injections, was obtained from Capital Lab Supplies CC, New Germany, South Africa, agents for Supelco.

Standard solutions

- A composite standard of 1 mg/L of GSM and 2-MIB in methanol was prepared from the commercial methanol solutions of Geosmin stock 2 mg/mL and the 2-MIB stock 10 mg/mL.
- A working composite standard of 1 $\mu\text{g}/\text{L}$ in MQ water was prepared from the above composite standard. The stability of these standards has been reported previously [2].
- Aqueous standards, of concentration: 5, 30, 100 and 200 ng/L,

were prepared daily in MQ water, from the working composite standard.

Purge-and-trap method

A commercial OI Analytical purge-and-trap sample concentrator (Eclipse, Model 4660) coupled to a OI Analytical 50 Vial Auto sampler (Model 4551-A), was used, which automatically dispensed 5-25 mL aliquots of water sample into a 25 mL fritted purging device (sparger). A transfer line was connected from the concentrator directly to the carrier gas line on the split/splitless inlet of the GC, and was insulated to prevent development of a cold spot. Samples were kept chilled at $\pm 4^\circ\text{C}$ in the Auto sampler rack, via a Constant Temperature Circulator (CARON, Model 2050), that was connected to the Auto sampler.

After pre-heating the sample (25 mL) for 1.0 min, target compounds were purged from the aqueous sample, at 80°C , by the use of helium gas, at a purge flow of 40 mL/min, and were adsorbed onto a Tenax trap at room temperature. During the purge cycle, the trap temperature was set at 20°C , and the water management fitting was set at a temperature of 120°C .

After the adsorption stage, the helium was passed through the trap for 3.0 min to remove any water trapped on the sorbent (dry purge). The trap temperature was then pre-heated to 175°C (pre-heat step). The trap temperature was then further raised to 180°C . The trapped analytes were desorbed from the trap, set at 180°C , with helium gas, for 3 min (desorb stage), and entered the injection port of the GC, via a transfer line, set at 110°C .

A “wash” cycle for the purge-and-trap extractor and auto sampler, between GC-MS runs, was used for cleaning, and the trap was baked at 200°C for 15 min. These processes were adequate to reduce carryover of the technique to a negligible level. Detailed conditions are presented in Table 2.

GC-MS conditions

GC-MS Analyses were performed on an Agilent Technologies

Entry	Sample concentration technique	2-MIB <i>m/z</i>	GSM <i>m/z</i>	^a Sample ($^\circ\text{C}$)	^a Ref.
1	Closed loop stripping analysis	95, 107, 135	111, 112, 125, 182	25	[17]
2	Dynamic headspace	95, 107, 108, 110	97, 111, 112, 125	85	[18]
3	Static headspace	95, 107, 108	111, 112, 125	80	[19]
4	Headspace-thermal desorption	95, 108, 135	112,182	45	[20]
5	Liquid-liquid extraction	95, 110, 150	112, 125, 149	ambient	[21]
6	Liquid-liquid extraction	95, 108, 110	108, 112	70	[22]
7	Liquid-liquid extraction	95, 108, 124, 151	111, 112	ambient	[23]
8	Solid phase microextraction	95, 107, 108, 135	97, 112, 125, 149	65	[24]
9	Solid phase microextraction	95, 107	112, 125	65	[25]
10	Solid phase microextraction	95	112	65	[26]
11	Headspace solid phase microextraction	95	112	65	[27]
12	Purge-and-trap	95, 135, 168	112, 126, 182	22	[3]
13	Purge-and-trap	95, 108	112, 125	ambient	[12]
14	Purge-and-trap	95.1, 168	^c	ambient	[10]
15	Purge-and-trap	95, 107, 108, 135	97, 111, 112, 125	80	[11]
16	Purge-and-trap	95, 107, 108, 135	97, 111, 112, 125	60	[2]

^a temperature

^b References

^c GSM not assayed

Table 1: Mass fragments used for 2-MIB and GSM by GC-MS analysis in SIM mode, and sample extraction temperature.

7890A GC Systems, equipped with an electronically controlled split/splitless injection port, set at 200°C, a split liner and interfaced to an Agilent Technologies 5975C Inert MSD, in electron ionization (EI) mode, with Triple-Axis Detector. The septum purge vent of the GC was plugged to prevent further losses of target analytes. The conventional GC separation employed a HP5-MS column, of dimensions 30 m x 0.25 mm x 0.25 µm. Helium was the carrier gas, at flow rate of 1.2 mL/min. The oven was set at 40°C (3 min) and raised to 160°C (held for 2 min) at 5°C/min. The oven temperature was then further raised to a final temperature of 280°C (held for 5 min) at 20°C/min; total run time was 22 min. Electron ionization was performed at 70eV, the source temperature and the GC interface temperature were set at 230°C. The emission current was 34.6 µA and the detector voltage was set at the EMV Mode, with a Gain Factor of 25.

For SIM mode, each target compound was monitored and identified using three ions: m/z 95, 107, 108 for 2-MIB, and 111, 112, 125, for GSM respectively. The retention windows were 8.0-13.0 min and 13.5-16.0 min, for 2-MIB and GSM, respectively. Acquisition was also performed in scan mode, from 40 to 300 amu, for identification purposes. Detailed conditions are presented in Table 2.

- The upgraded 6 mm drawout plate (part number: G2589-20045) was obtained from Chemetrix (Pty) Ltd. (Halfway House, South Africa), local agents for Agilent Technologies.

- The NIST mass spectra were also accessed from the MSD Chemstation Data Analysis Software for confirmation of target analyte identification.
- The 1 mg/L methanol standard was used for liquid auto sampler injections. Detailed conditions are presented in Table 2.

Results and Discussion

Development and optimization of analytical aspects of method

Preliminary development work was done by OI Analytical as a pre-purchasing agreement that the new Eclipse sample concentrator is able to analyze these taste-odorants at levels down to our specific requirements of ±10 ng/L.

Our earlier work on the Tekmar-Stratum purge-and-trap sample concentrator for this analysis [2] was also utilized as an initial guide regarding the various method parameters.

A combination of standards made in organic solvent (methanol), injected by the GC liquid auto sampler, and in water, extracted by the purge-and-trap, was both used to optimize all parameters.

Optimization of the GCMS conditions: Retention times were established by both auto sampler injections of suitable liquid methanol

Variable	Value	Variable	Value
Purge-trap:			
Trap	Tenax	GC start	Start of desorb
Valve oven temperature	140°C	Desorb preheat temperature	175°C
Transfer line temperature	110°C	Desorb temperature	180°C
Sample mount temperature	90°C	Desorb drain	On
Sample preheat time	1 min	Desorb time	3 min
Sample temperature	80°C	Bake time	15 min
Purge time	11 min	Bake temperature	200°C
Purge temperature: Trap	20°C	Bake flow	200 mL/min
Purge flow	40 mL/min	Water management fitting : At Bake: temperature	240°C
Water management fitting: At Purge: temperature	120°C	Water management fitting: At Desorb: temperature	0°C
		Dry purge time	3.00 min.
GC-MS:			
GC		MS	
Oven:		Mode	EI/SIM, SCAN
Helium gas flow	1.2 mL/min	Electron energy	70 ev
Initial temperature/time	40°C/3 min	Emission current	34.6 µA
Ramp rate 1	5°C/min	EMV mode	Gain Factor/Atune
Final temperature 1/final time 1	160°/2 min	Gain Factor	25.00
Ramp rate 2	20°C/min	Transfer line temperature	230°C
Final temperature 2/final time 2	280°C/5 min	Source temperature ^b	230°C
Injector:		Quadrupole temperature	150°C
Heater	200°C	Solvent delay ^c	7.5 min
Pressure	9.1473 psi		
Total flow	4.8 mL/min	Scan parameters:	
Septum purge flow	Off	Low mass	40.0
Injector mode	Pulsed (23 psi), Split (3 min)	High mass	200.0
Injection volume	1 µL ^a	Threshold	0
Split ratio	3:1	Sample #	2 A/D samples 4
Split flow	3.6 mL/min		

^a Liquid auto sampler

^b Default; varied up to 350°C maximum

^c SIM mode

Table 2: Purge-and-trap and GC-MS instrument parameters.

composite standards that were analyzed by standard GC-MS (scan and SIM modes) and by comparison with library spectra from the Chemstation software. The aqueous composite standards (5, 30, 100 and 200 ng/L) were used for purge-and-trap extractions.

Injection technique: A pulsed split injection technique was chosen to maximize efficient transfer of the analytes to the GC column, as per earlier work [2]. It was noted that a low split ratio of 3:1 was found to give maximum peak area response for the target analytes. The injection port temperature was set at 200°C [2].

The transfer line was connected directly to the carrier gas line on the split/splitless inlet and insulated to prevent development of a cold spot; the septum purge vent was plugged to prevent further losses.

Mass spectrometer acquisition: The selected ion monitoring mode (SIM) was chosen [2]. Method development studies included comparison of three ions per analyte: m/z 95, 107, 108 for 2-MIB, and 111, 112, 125 for GSM. Ions m/z 135, for 2-MIB, and 149, previously used [2] were omitted to enhance sensitivity in the SIM mode.

Qualitative identification was based on retention time analysis. Mass spectral verification was done by comparison of relative abundance values of the quantification and qualification ions to the same values obtained from the standard samples and the NIST reference spectra for target analytes 2-MIB and GSM.

Optimization of the purge-and-trap parameters:

- **Sample size:** Most USEPA methods are designed for 5 mL samples. A 25 mL sample aliquot was used [2]; a 25 mL fritted sparge vessel was supplied with the sample concentrator on commission of the new equipment, for more efficient purging.
- **Effect of trap:** The #7 Tenax trap was supplied by the vendor, and was used; our earlier work [2] was also considered.
- **Effect of purge cycle temperature:** The recommended temperature of 80°C, by the vendor, was initially used to heat samples during the purge cycle.
- **Effect of purge pre-heat temperature:** Improved peak area

response was noted by use of a sample preheat time of 1.0 min, compared to 0 min.

- **Effect of purge cycle time:** A purge time of 11 minutes was recommended by OI Analytical, and its variation effect was not tested.
- **Effect of purge cycle flow of helium gas:** The flow rate of 40 mL/min was used as per recommendation by the vendor.
- **Effect of trap desorb time:** A time of 3 min was found to be optimum.

The NIST reference mass spectrum for 2-MIB and GSM

To confirm unambiguous target peak identification for the purge-and-trap analysis, we obtained the reference mass spectra for 2-MIB and GSM from NIST [29]. The CAS number for 2-MIB is: 2371-42-8, with Formula $C_{11}H_{20}O$ and Molecular weight of 168; the exact mass is 168.151415. The CAS number for GSM is: 19700-21-1, with Formula and Molecular $C_{12}H_{22}O$ weight of 182; the exact mass is 182.167066.

The reference NIST mass spectrum [29] for 2-MIB is shown in Figure 1. Table 3 shows the relative abundance of the mass fragments for 2-MIB: the base peak is mass fragment m/z 95, with molecular ion m/z 168 (2%). The observed relative abundances for mass fragments 107, 108 and 150, are 25%, 21% and 7% respectively.

The corresponding NIST mass spectrum [29] for GSM is shown in Figure 2. Table 4 shows the relative abundance of the mass fragments for GSM: the base peak is mass fragment m/z 112, with molecular ion 182 (7%). The observed relative abundances for mass fragments 111, 125 and 182, are 23%, 14% and 7%.

Water management in the eclipse sample concentrator

Geosmin and 2-MIB both have retention times exceeding the portion of the chromatogram where excess water may cause problems. Failure to use an efficient water removal configuration could have an effect on resultant peak shape, peak symmetry, and more importantly, a detrimental effect on the mass selective detector.

The hydrophobic Tenax Number #7 trap and a 3-minute Dry Purge time were recommended for this application by OI Analytical

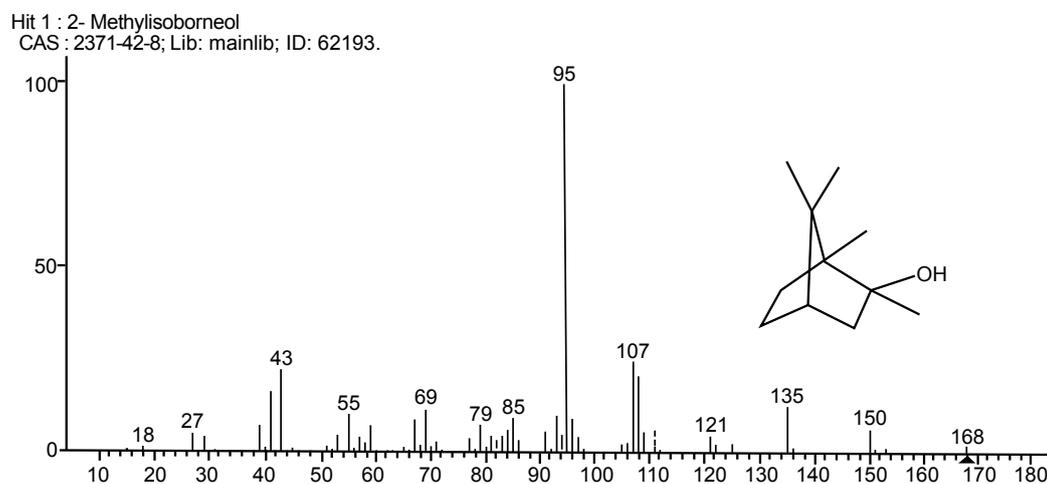


Figure 1: NIST reference mass spectrum of 2-MIB.

<i>m/z</i>	Abundance (%)	<i>m/z</i>	Abundance (%)	<i>m/z</i>	Abundance (%)
15	0.90	78	0.80	126	0.30
17	0.30	79	7.31	127	0.20
18	1.40	80	1.40	133	0.10
26	0.30	81	4.30	134	1.01
27	5.01	82	3.00	135	12.71
28	0.90	83	4.20	136	1.30
29	4.51	84	5.91	137	0.10
30	0.10	85	9.21	139	0.20
31	0.60	86	3.30	149	0.10
32	0.10	87	0.20	150	6.51
38	0.30	89	0.10	151	0.90
39	6.91	91	5.51	152	0.10
40	1.20	92	0.90	153	1.40
41	16.22	93	9.81	154	0.20
42	1.10	94	4.81	168 (M ⁺)	2.00
43	22.22	95	100.000	169	0.30
44	0.60	96	8.81		
45	1.00	97	4.00		
50	0.50	98	1.00		
51	1.40	99	0.30		
52	0.70	103	0.20		
53	4.51	104	0.10		
54	0.90	105	2.00		
55	10.21	106	2.70		
56	1.00	107	24.53		
57	4.00	108	20.52		
58	2.50	109	5.51		
59	7.11	110	14.62		
60	0.30	111	6.21		
62	0.20	112	0.80		
63	0.50	113	0.70		
64	0.20	114	0.10		
65	1.70	115	0.20		
66	0.70	117	0.10		
67	8.51	119	0.60		
68	2.00	120	0.40		
69	11.41	121	4.51		
70	1.60	122	2.20		
71	2.60	123	0.50		
72	0.60	124	0.20		
77	3.50	125	2.50		

Table 3: NIST Reference mass spectral analysis 2-MIB: Mass fragments and relative abundances.

to promote the removal of water. The recommended settings for the Water Management Fitting are listed in Table 2: Purge 120°C, Desorb 0°C, and Bake 240°C. A fair amount of water can still enter the GC due to the heated purge and the low split ratio.

The actual specifications for the Eclipse water management [30,31] claims that the system eliminates all but approximately 0.25 µL (0.063 µL/min) of trapped desorb water (> 96% water removal).

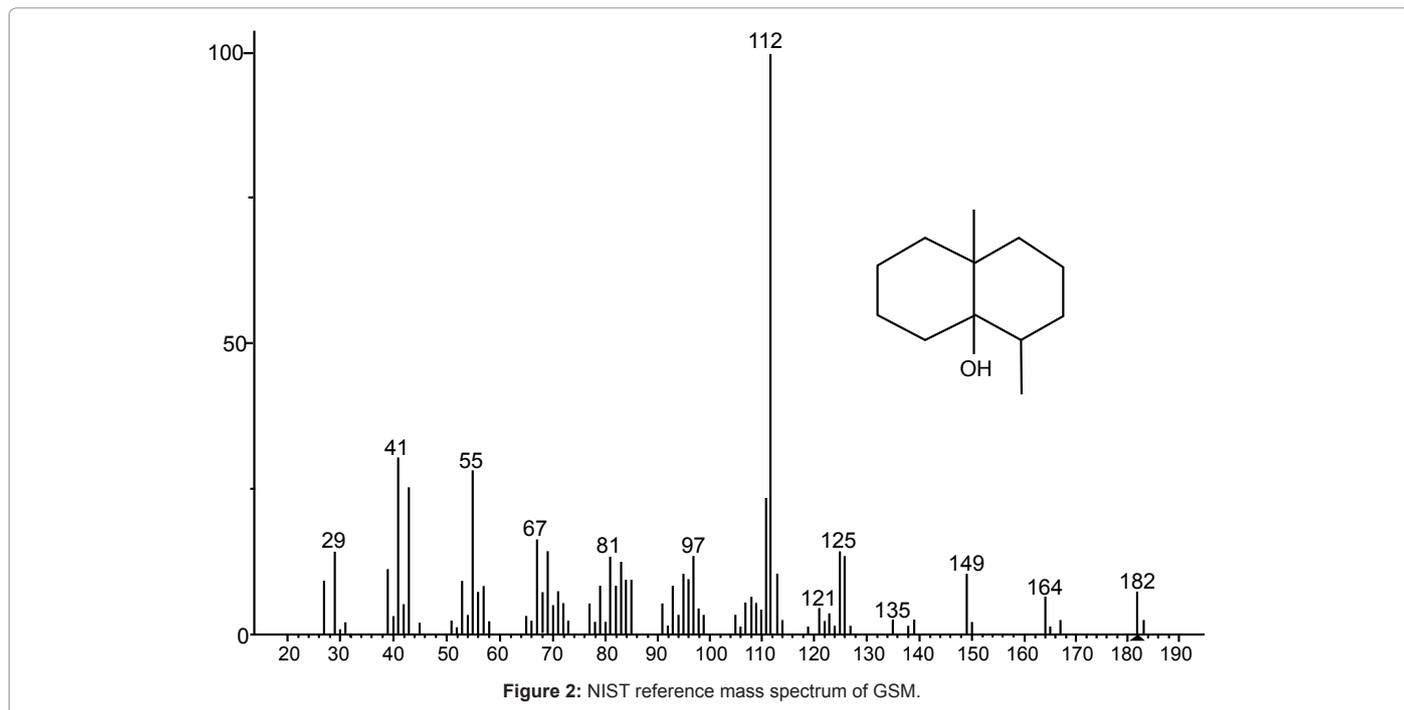
Use of an upgraded drawout plate in the ion source

Headspace is another commonly used sample concentration technique for analysis of taste-odorants, coupled to MS detection [18-20,27]. The Agilent mass selective detectors, either the 5973, or the more recent 5975C model, has a standard 3 mm drawout plate in the ion source. To date, there is just one report [19] on the positive effect of the use of a 6 mm drawout plate, as opposed to the conventional 3 mm drawout plate, on the more efficient removal of water, and on

the resultant ratio of the ions *m/z* 95 and 107 for 2-MIB in headspace analysis. With the standard 3 mm plate, it was observed that *m/z* 107 was the base peak; the 6 mm plate was effective in reducing water from the ion source and the resulting mass spectrum was then identical to the NIST reference spectrum, with *m/z* 95 as the base peak [19].

However work on the use of a 6 mm drawout plate, on the relative ratio of mass fragments *m/z* 95, 107 for 2-MIB, GSM, or other taste-odorants, and its subsequent effects, has not been reported for analysis of taste odorants specifically by the purge-and-trap sample concentration technique.

In our initial method optimization, prior to our discovery that 2-MIB dehydrates to 2-M-2-B, our intention was to investigate the effect of the 6 mm drawout plate, based on its successful application in headspace [19] on the relative ratios of *m/z* 95 and 107 for 2-MIB. The idea of baking the source to remove water after our observations on



<i>m/z</i>	Abundance (%)	<i>m/z</i>	Abundance (%)
27	2.70	95	10.01
29	14.01	96	9.01
30	1.00	97	13.01
31	2.00	98	4.00
39	11.01	99	3.00
40	3.00	105	3.00
41	30.03	106	1.00
42	5.01	107	5.01
43	25.03	108	6.01
45	2.00	109	5.01
51	2.00	110	4.00
52	1.00	111	23.02
53	9.01	112	100.00
54	3.00	113	10.01
55	28.03	114	2.00
56	7.01	119	1.00
57	8.01	121	4.00
58	2.00	122	2.00
65	3.00	123	3.00
66	2.00	124	1.00
67	16.02	125	14.01
68	7.01	126	13.01
69	14.01	127	1.00
70	5.01	135	2.00
71	7.01	138	1.00
72	5.01	139	2.00
73	2.00	149	10.01
77	5.01	150	2.00
78	2.00	164	6.01
79	8.01	165	1.00
80	2.00	167	2.00
81	13.01	182 (M+)	7.01
82	8.01	183	2.00
83	12.01		
84	9.01		
85	9.01		
91	5.01		
92	1.00		
93	8.01		
94	3.00		

Table 4: NIST Reference mass spectral analysis of GSM: Mass fragments and relative abundances.

the erratic relative ratios observed for m/z 95 and 107 prompted us to investigate the effect of elevated source temperature on these ion ratios and precision, for GSM as well.

Use of high ion source temperature

The default ion source temperature for electron impact operation is 230°C. This temperature has a wide range of applicability and has been the default for this reason. The new 5975C MSD ion source is fully configured for use up to 350°C.

Increasing the ion source temperature has advantages and disadvantages [32] and should be carefully considered and tested in applications before implementation.

However work on source temperature variation on the relative ratio of m/z 95, 107, 108 for 2-MIB, GSM, or other taste-odorants, and its subsequent effects, has not been reported for analysis of taste odorants, specifically by the purge-and-trap sample concentration technique.

The mass spectrum of 2-MIB and GSM by liquid auto sampler injection

The full scan and SIM mode spectra, using the 1 mg/L composite standard in methanol, were acquired using the conditions in Table 2.

Table 5 shows the commonly used mass fragment ratios, for m/z 107/95, 108/95, for 2-MIB, at source temperatures 230-350°C obtained with the standard 3 mm drawout plate, and with the upgraded 6 mm plate. All reported ratios are based on data from a single injection, for each mode, run on the same day.

For the 3 mm plate: The peak for 2-MIB elutes at 11.96 min for both the scan and SIM modes. At all source temperatures, the observed base peak is m/z 95, for both modes, consistent with the NIST reference spectrum for 2-MIB. The relative ratios of m/z 107/95 and 108/95, are 83% and 31 %, and 82% and 33%, for the scan and SIM

modes, respectively. The corresponding ratios for the NIST reference spectrum, in scan mode, are 25% and 21% respectively. It is evident that a much higher (about three times) ratio for m/z 107/95 is obtained on the current Agilent MS equipment. It must be noted that the NIST spectrum for 2-MIB was recorded on a magnetic sector instrument [29]. There is also a noticeable decrease in the relative abundance of the molecular ion m/z 168 with increasing source temperature, from 1.9 to 0.5%.

For the 6 mm plate: The peak for 2-MIB elutes at 11.96 min for both scan and SIM modes (Figure 3). At all source temperatures, the observed base peak is m/z 95, for both modes, consistent with the NIST reference spectrum for 2-MIB. The relative ratios of m/z 107/95 and 108/95, are 30% and 23 % and 31% and 24%, for the scan and SIM modes, respectively. The corresponding ratios for the NIST reference spectrum are 25% and 21% respectively. There is also a noticeable decrease in the relative abundance of the molecular ion m/z 168 with increasing source temperature, from 2.1% to 0.7%.

Unlike the ratios obtained with the 3 mm plate, the ratios are fairly similar to that of the NIST spectrum. This is somewhat unexpected as there is no significant amount of water from the liquid injection of a methanol sample (1mg/L standard).

Table 6 shows the commonly used mass fragment ratios, for GSM, for m/z 111, 112, 125 and 182, at source temperatures 230-350°C obtained with standard 3 mm drawout plate, and with the upgraded 6 mm plate. All reported ratios are based on data from a single injection, for each mode, run on the same day.

For the 3 mm plate: The peak for GSM elutes at ± 15.18 min. (Figure 3) for both the scan and SIM modes. At all source temperatures, the observed base peak is m/z 112, for both modes, consistent with the NIST reference spectrum for GSM 2-MIB. The mean relative ratios of m/z 111/112, 125/112 and 182/112 are 25%, 15% and 2% in the scan

DP ^a	Source ^b (°C)	Scan t _r (min)	Scan % m/z 107/95	Scan % m/z 108/95	Scan % m/z 150/95	Scan % m/z 168/95	SIM t _r (min)	SIM % m/z 107/95	SIM % m/z 108/95
3 mm	230	11.96	96	34	22	1.9	11.96	94	35
	250	11.96	87	33	19	1.7	11.96	78	35
	270	11.96	82	32	17	1.5	11.96	85	34
	290	11.96	83	32	15	1.0	11.96	80	33
	310	11.96	75	31	13	0.8	11.96	77	32
	330	11.96	77	30	12	0.6	11.96	78	31
	350	11.96	83	27	5	0.5	11.96	82	30
Mean			83	31	15	1.1		82	33
SD			7	2	5	0.5		6	2
RSD%			8.47	7.56	37.00	47.54		7.11	6.00
6 mm	230	11.96	31	23	23	2.1	11.96	36	27
	250	11.96	33	24	22	1.4	11.96	35	26
	270	11.96	32	24	22	1.6	11.96	33	26
	290	11.96	30	23	17	1.1	11.96	31	24
	310	11.96	27	22	13	0.9	11.96	28	23
	330	11.96	26	22	13	0.8	11.96	28	22
	350	11.96	26	21	7	0.7	11.96	28	21
Mean			30	23	17	1.2		31	24
SD			3	1	6	0.5		3	2
RSD%			9.84	4.42	36.17	38.95		11.18	8.83

^a Drawout plate
^b temperature

Table 5: Scan and SIM mode mass spectral analysis of 2-MIB by liquid auto sampler injection.

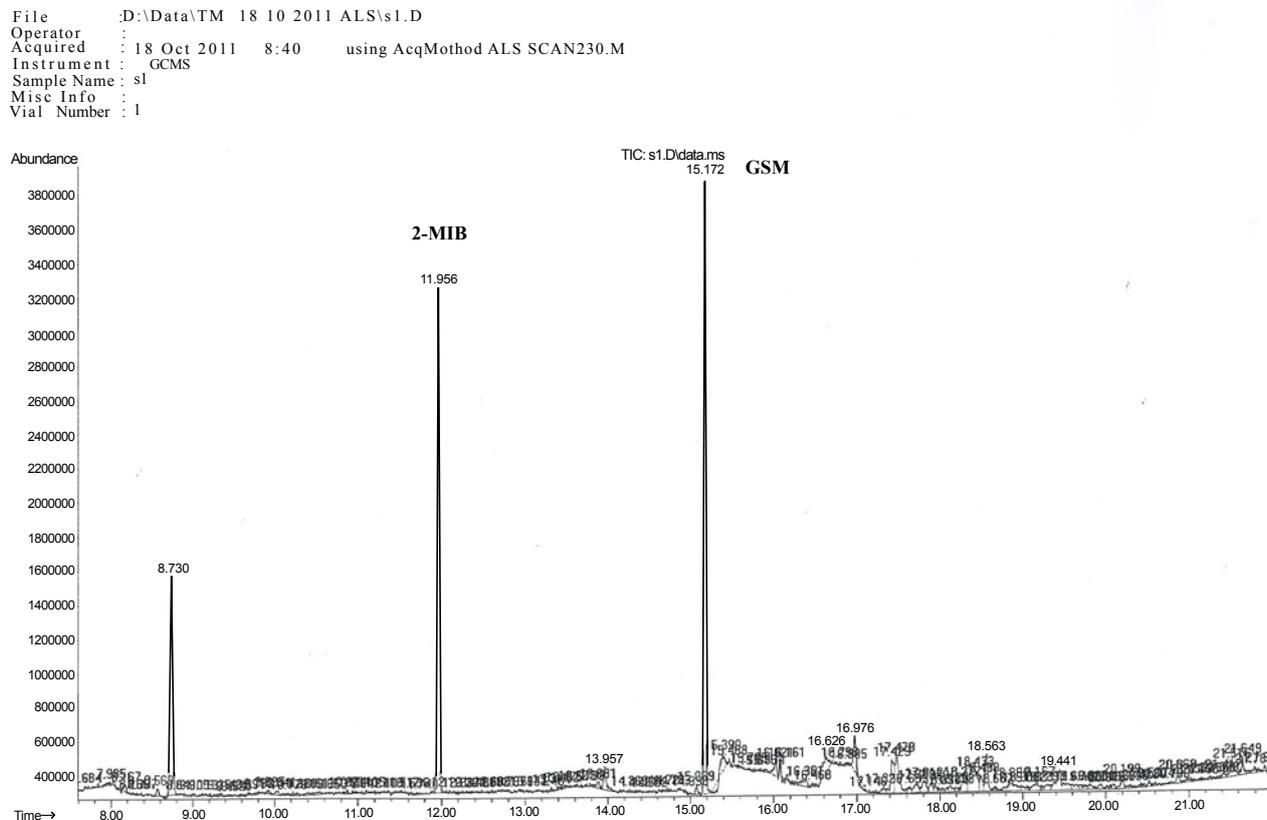


Figure 3: Scan mass spectrum of 1 mg/L composite standard of 2-MIB/GSM by auto sampler liquid injection.

Drawout plate (mm)	°Source (°C)	Scan t _r (min)	Scan % m/z 111/112	Scan % m/z 125/112	Scan % m/z 182/112	SIM t _r (min)	SIM % m/z 111/112	SIM % m/z 125/112
3	230	15.17	22	15	3	15.18	23	15
	250	15.17	23	15	3	15.18	23	15
	270	15.18	23	15	2	15.18	24	15
	290	15.18	24	16	2	15.18	26	16
	310	15.17	25	15	1	15.18	26	16
	330	15.18	28	15	1	15.18	27	16
	350	15.18	26	17	1	15.18	28	16
Mean		15.18	25	15	2	15.18	25	16
SD		0.00	2	1	1	0.00	2	1
RSD%		0.01	7.58	4.61	41.17	0.00	7.09	4.30
6	230	15.17	22	14	3	15.17	22	15
	250	15.17	24	14	2	15.17	24	15
	270	15.17	24	14	2	15.17	24	15
	290	15.18	24	15	2	15.18	25	15
	310	15.17	26	16	1	15.18	26	15
	330	15.18	27	16	1	15.18	27	16
	350	15.18	28	16	1	15.18	28	16
Mean		15.17	25	15	2	15.18	25	15
SD		0.01	2	1	1	0.01	2	1
RSD%		0.04	7.75	5.81	36.40	0.04	7.13	3.63

*temperature

Table 6: Scan and SIM mode mass spectral analysis of GSM by liquid auto sampler injection.

mode. The corresponding ratios for the NIST reference spectrum, is 23%, 14 and 7% respectively. The mean relative ratios of m/z 111/112 and 125/112 are 25% and 16% in the SIM mode. The corresponding ratios for the NIST reference spectrum, is 23% and 14% respectively. There is also a noticeable decrease in the relative abundance of the molecular ion m/z 182 in scan mode, with increasing source temperature, from 3% to 1%.

For the 6mm plate: The peak for GSM elutes at ± 15.18 min for both the scan and SIM modes. At all source temperatures, the observed base peak is m/z 112, for both modes, consistent with the NIST reference spectrum for GSM. The mean relative ratios of m/z 111/112, 125/112 and 182/112 are 25, 15% and 2% in the scan mode. The corresponding ratios for the NIST reference spectrum, is 23%, 14 and 7% respectively. The mean relative ratios of m/z 111/112 and 125/112 are 25% and 15% in the SIM mode. The corresponding ratios for the NIST reference spectrum, is 23% and 14% respectively.

There is also a noticeable decrease in the relative abundance of the molecular ion m/z 182 in scan mode, with increasing source temperature, from 3% to 1%.

These results confirm the elution times of 2-MIB and GSM, at ± 11.96 min and ± 15.18 min, using liquid auto sampler, coupled to GC-MS, under the instrumental conditions employed.

Initial results after baking source at 300°C, by purge-and-trap, with 3 mm drawout plate

At the very outset, with the standard (default) factory fitted 3mm drawout plate, we initially observed apparent erratic elution times for 2-MIB, at ± 12 and ± 9 min. The source temperature was initially increased to 300°C for ± 3 hr. in an attempt to remove any suspected water from the ion source [19]. A composite standard of 100 ng/L was then analyzed at source temperatures of 230-290°C, in both scan and SIM modes. Results are listed in Table 7 and 8, based on a single sample extraction per source temperature. Figure 4 shows various peaks, of note the two at 11.987 min and 15.190 min, at a source temperature of 230°C.

With this particular run, for the major peak at 11.96 min for both scan and SIM modes, the mean ratios for m/z 107/95 and 108/95 are 39%, 31% and 37, 33%, for the scan and SIM modes respectively, which compares fairly well with the corresponding 2-MIB NIST reference spectrum ratios of 25 and 21% respectively, and the 6 mm plate, (mean retention time 11.96 min), by liquid auto sampler injection (Table 5). The corresponding ratios obtained by us on the Agilent 5975C MS, with the 3 mm drawout plate, were 83% and 31% (mean retention time 11.96 min) (Table 5). This data indicates that 2-MIB elutes at ± 11.96 min under the purge-and-trap conditions used. For all scan spectra, at all source temperatures tested, there was no observable peak at ± 9.19 min.

There is also another major peak eluting at 15.19 min for both scan and SIM modes. The mean ratios for m/z 111/112, 125/112 and 182/112 are: 25%, 16% and 2%, for scan mode, and 25% and 15% by SIM mode (Table 8). This data indicates that GSM elutes at ± 15.19 min. under the purge-and-trap conditions used.

However, all subsequent investigations at source temperatures exceeding 230°C, up to the maximum 350°C, with both 3 and 6 mm were not successful in reproducing the results obtained for 2-MIB, in Table 7 (Figure 4).

Mass spectral analysis of composite, 200 ng/L aqueous standards by purge-and-trap

Table 9 summarizes the scan mode mass spectral analysis with the standard 3 mm drawout plate, for the peak at 9.18 min. (Figure 5).

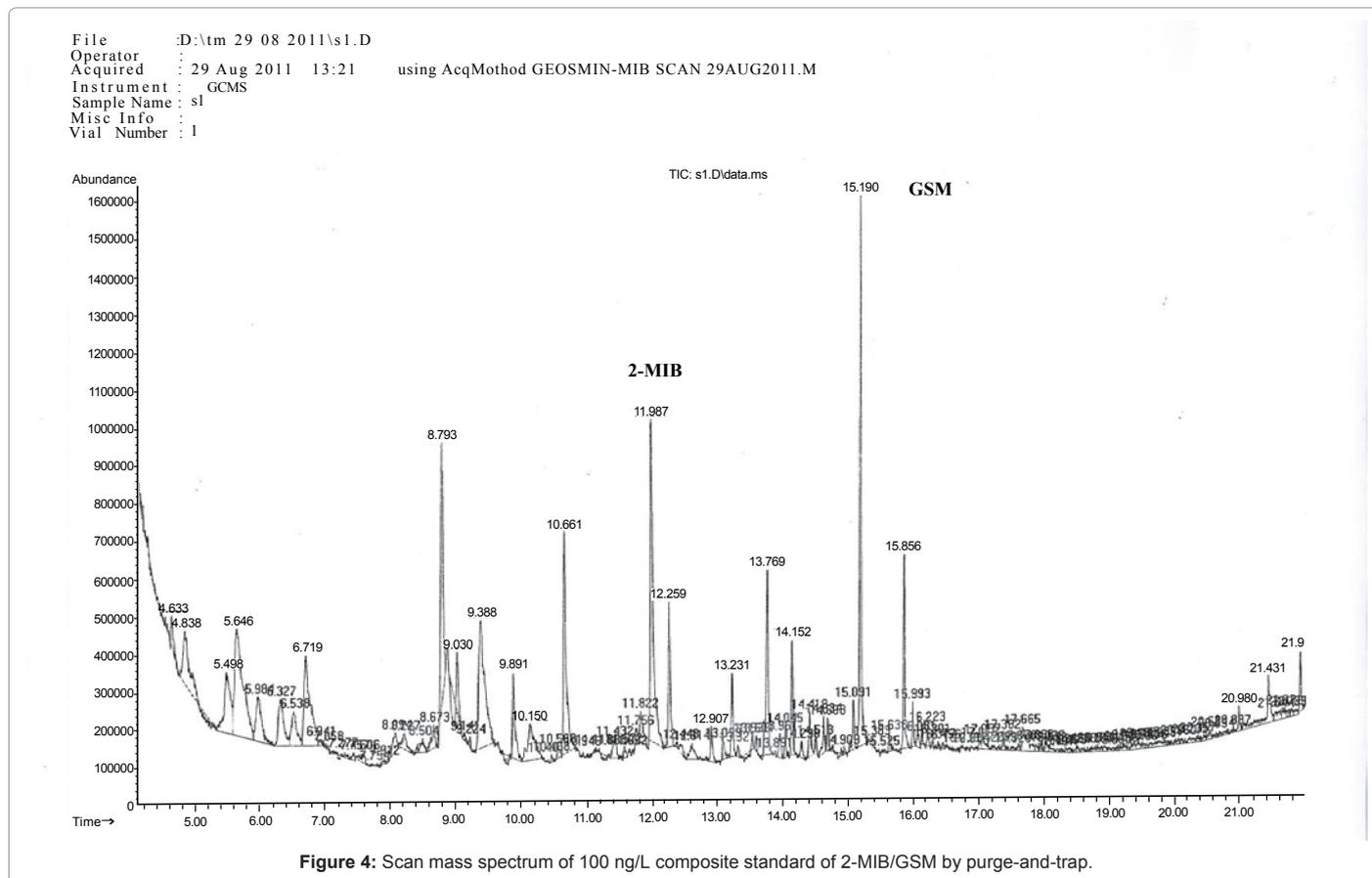
At all source temperatures, the observed base peak for this signal is m/z 107, inconsistent with the NIST reference spectrum for 2-MIB, where the base peak is m/z 95, and also the liquid auto sampler injection (base peak m/z 95). The relative ratios of m/z 107/95 and 108/95 are on average, 289% and 61% respectively. The corresponding ratios for the NIST reference spectrum is 25% and 21% respectively; 83% and 31 % were obtained by liquid auto sampler injection.

The relative ratios of m/z 95/107, 108/107 and 150/107, averaged

Mode	Source temperature (°C)	t _r (min)	% m/z 107/95	% m/z 108/95	% m/z 150/95	% m/z 168/95	t _r (min)	% m/z 107/95	% m/z 108/95
Scan	230	11.99	42	28	8	1.8	-	-	-
	250	11.99	33	27	8	1.7	-	-	-
	270	11.99	35	32	4	2.2	-	-	-
	290	11.99	44	36	6	0.8	-	-	-
Mean		11.99	39	31	7	1.6	-	-	-
SD		0	6	4	2	0.6	-	-	-
RSD%		0	14.48	12.65	24.62	35.41	-	-	-
SIM	230	11.99	38	38	^a		9.16	446	81
	250	11.99	37	30	^a		9.16	255	56
	270	11.99	35	31	^a		9.16	279	62
	290	11.99	44	32	^a		9.16	275	51
Mean		11.99	37	33		9.16	314	63	
SD		0	6	3		0	89	13	
RSD%		0	14.80	10.20		0	28	20.95	

^a m/z 150 not monitored in SIM mode

Table 7: Initial scan and SIM mass spectral analysis of 2-MIB by purge-and-trap: 100 ng/L composite standard in MQ water.



Mode	Source temperature (°C)	t _r (min)	% m/z 111/112	% m/z 125/112	% m/z 182/112
Scan	230	15.20	25	15	2.1
	250	15.19	23	16	2.0
	270	15.19	27	17	1.4
	290	15.19	25	15	1.6
Mean		15.19	25	16	1.8
SD		0.00	2	1	0.4
RSD%		0.01	6.79	7.66	20.22
SIM	230	15.19	24	15	^a
	250	15.19	25	16	^a
	270	15.19	26	15	^a
	290	15.19	27	16	^a
Mean		15.19	25	15	
SD		0.00	1	1	
RSD%		0.00	5.04	3.84	

^am/z 182 not monitored in SIM mode

Table 8: Initial scan and SIM mass spectral analysis of GSM by purge-and-trap: 100 ng/L composite standard in MQ water.

over all source temperatures, were: 35%, 21% and 15% respectively. The corresponding ratios for the NIST reference spectrum for 2-M-2-B are: 36%, 21% and 22% respectively. Except for source temperature 350°C, the average quality fit was 75%, and identification gave 2-M-2-B, using the GCMS Chemstation Software. Table 10 summarizes the scan mode mass spectral analysis for the peak at 15.18 min, with the standard 3 mm drawout plate.

At all source temperatures, the observed base peak for this signal is m/z 112, consistent with the NIST reference spectrum for GSM, where the base peak is m/z 112 (7%), and also the liquid auto sampler injection (base peak m/z 112). The relative ratios of m/z 111/112, 125/112 and 182/112, are, on average, 25%, 15%, and 2% respectively. The corresponding ratios for the NIST reference spectrum is 23%, 14% and 7% and respectively; 25%, 15% and 2% were obtained by liquid auto sampler injection.

Source (°C)	t _r (min)	% m/z 107/95	% m/z 108/95	% m/z 95/107	% m/z 108/107	% m/z 150/107	Quality ^c (%)	id ^d
230	9.176	286	67	35	24	24	62	2-M-2-B
250	9.172	275	64	36	23	21	94	2-M-2-B
270	9.165	309	60	32	19	18	62	2-M-2-B
290	9.163	293	58	34	20	16	62	2-M-2-B
310	9.165	288	60	35	21	15	94	2-M-2-B
330	9.163	313	64	32	21	13	76	2-M-2-B
350	9.176	276	61	36	22	12	62	2-M-2-B
Mean	9.176	292	62	34	21	17	73	
SD	9.172	15	3	2	2	4	15	
RSD%	9.165	5.13	5.31	5.04	7.89	26.06	20.70	

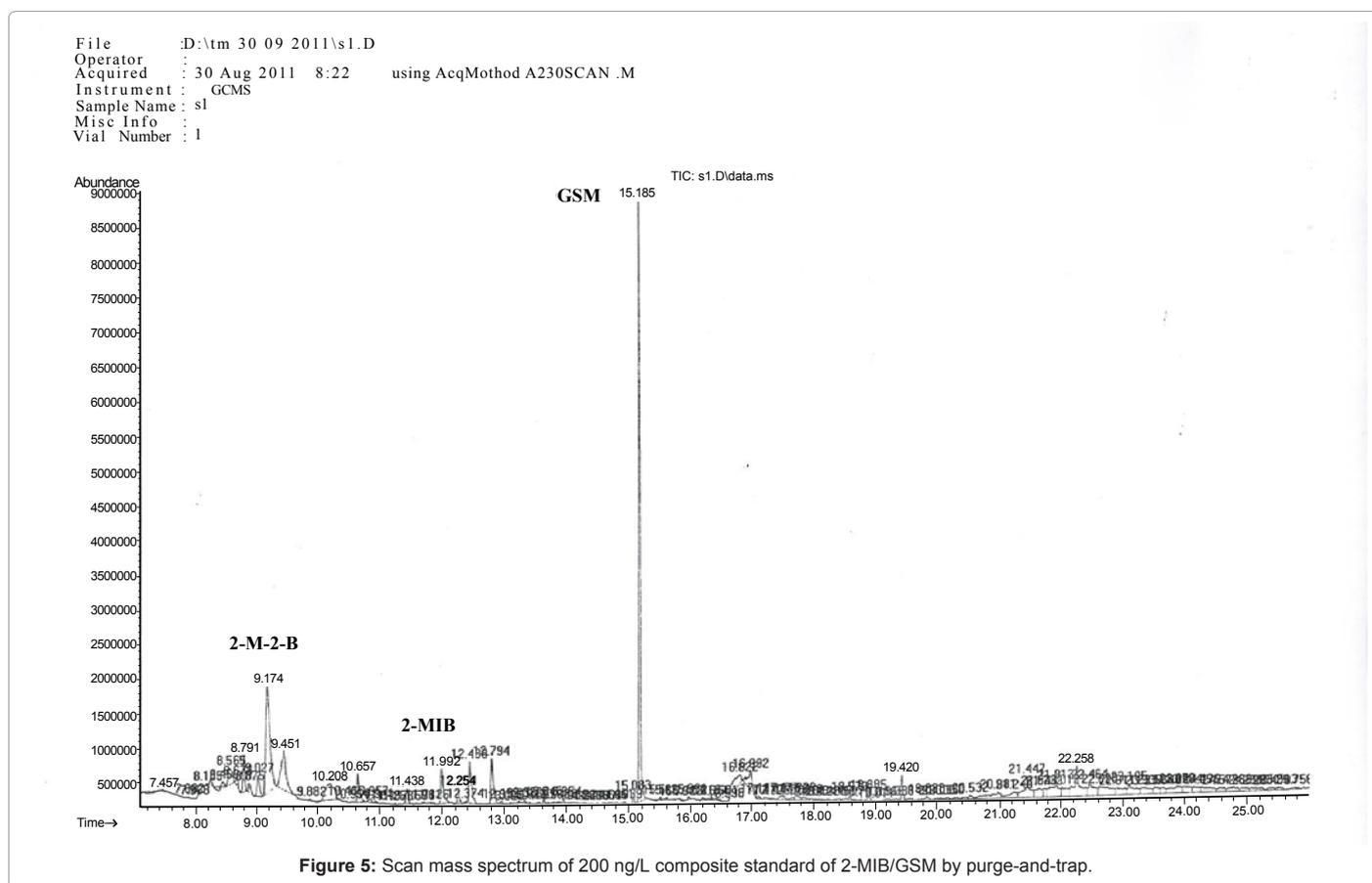
^a3 mm drawout plate

^b200 ng/L in MQ water

^c as per the GCMS Chemstation software library

^d Compound name/identification as per GCMS Chemstation software

Table 9: Scan^a mass spectral analysis of composite 2-MIB/GSM standard^b by purge-and-trap: 200ng/L.



Further examination of these spectra at all source temperatures indicated presence of a minor peak at ± 12.00 min. However, the mass spectral analysis indicated that this peak was not 2-MIB at all source temperatures.

The relatively high 200 ng/L concentration of this aqueous composite standard is considered adequate to allow fairly accurate retention time determination and subsequent identification of the target taste-odorants using purge-and-trap/GCMS. Based on the NIST reference mass spectra, and the liquid auto sampler injections, the data indicate that under the purge-and-trap/GCMS conditions, 2-M-2-B

elutes at ± 9.18 min., and GSM elutes at ± 15.18 min.

Mass spectral Analysis of 30 ng/L aqueous standards by purge-and-trap

A low concentration, composite standard, of 5 ng/L was initially used. Due to the general observation that within-day (repeatability) precision is better with increased concentration as a result of increased signal response (peak area, peak height), a 30 ng/L composite in MQ water was then analyzed, using the standard 3 mm and the upgraded 6 mm drawout plate. Table 11 summarizes the mass spectral analysis of peaks eluting at ± 9 and ± 12 min.

Source (°C)	t _r (min)	% m/z 111/112	% m/z 125/112	% m/z 182/112
230	15.183	23	14	3
250	15.183	23	14	2
270	15.183	24	15	2
290	15.185	24	15	2
310	15.185	26	16	2
330	15.185	26	16	1
350	15.183	27	17	1
Mean	15.184	25	15	2
SD	0.001	2	1	1
RSD%	0.01	6.34	6.19	35.72

^a3 mm drawout plate

^b 200 ng/L in MQ water

Table 10: Scan^a mass spectral analysis of GSM standard^b by purge-and-trap: 200ng/L composite standard in MQ water.

Drawout plate	Source temperature	t _r (min)	% m/z 95/107	% m/z 108/107	^a Area 12/9 min	t _r (min)	2-MIB % m/z 107/95	2-MIB % m/z 108/95
3 mm	230	9.16	35	22	1.4	11.99	62	43
	250	9.17	35	21	2.0	11.99	68	43
	270	9.18	34	21	2.0	11.99	55	40
	290	9.18	34	21	1.7	11.99	73	35
	310	9.18	34	20	1.8	12.00	55	29
	330	9.18	31	20	2.4	12.00	65	33
	350	9.18	31	20	2.7	11.99	68	31
Mean		9.18	33	21	2.0	11.99	64	36
SD		0.01	2	1	0.4	0.01	7	6
RSD%		0.09	5.14	3.65	21.79	0.04	10.71	15.81
6 mm	230	9.19	21	14	2.2	12	64	20
	250	9.19	23	15	3.1	12	54	30
	270	9.18	33	19	3.7	11.99	46	31
	290	9.18	33	21	3.4	11.99	44	28
	310	9.19	35	21	3.8	11.99	41	25
	330	9.19	26	15	3.2	12	40	25
	350	9.19	30	20	3.9	12	39	16
Mean		9.19	29	18	3.3	12.00	47	25
SD		0.01	5	3	0.6	0.01	9	5
RSD%		0.05	18.94	17.24	17.49	0.05	19.42	21.66

^aRatio of : Area of peak at ± 12.0 min /area of peak at ± 9.2 min

Table 11: SIM mass spectral analysis of composite 2-MIB/GSM standard by purge-and-trap: 30 ng/L in MQ.

For the 3 mm plate: A major peak is observed at 9.17 min on average. At all source temperatures, the observed base peak for this signal is m/z 107, inconsistent with the NIST reference spectrum for 2-MIB, where the base peak is m/z 95, and also the liquid auto sampler injection (base peak m/z 95). The relative ratios of m/z 107/95 and 108/95 are on average, 299% and 62% respectively. The corresponding ratios for the NIST reference spectrum is 25% and 21% respectively; 83% and 31% were obtained by liquid auto sampler injection.

For the 6 mm plate: A major peak is observed at 9.19 min. At all source temperatures, the observed base peak is m/z 107, inconsistent with the NIST reference spectrum for 2-MIB, where the base peak is m/z 95, and also the liquid auto sampler injection (base peak m/z 95). The relative ratios of m/z 107/95 and 108/95 are on average 368% and 62% respectively. The corresponding ratios for the NIST reference spectrum for 2-MIB is 25% and 21% respectively; 83% and 31% were obtained by liquid auto sampler injection.

Further examination of these SIM spectra indicated a minor peak

at 11.99 min. The relative ratios of the peaks at 9.2 min and 11.99 min were determined, from the TIC of the SIM mode chromatogram. The relative ratios for m/z 107/95 and 108/95 were again determined for the peak at 11.99 min. The results are summarized in Table 11.

For the 3 mm plate: The mean ratios for the area response of the peak at ± 12.0 min as a fraction of the major peak at ± 9.2 min were observed to be approximately 2.0%. The mean relative ratios for m/z 107/95 and 108/95 were 64% and 36%, respectively, over the source temperature range 230-350°C. The corresponding ratios for the NIST reference spectrum for 2-MIB is 25% and 21% respectively; 83% and 31% were obtained by liquid auto sampler injection.

For the 6 mm plate: The mean ratio for the area response of the peak at 11.99 min as a fraction of the major peak at 9.17 was observed to be approximately 3%. The mean relative ratios for m/z 107/95 and 108/95 were 47% and 25% respectively, over the source temperature range 230-350°C. The corresponding ratios for the NIST reference spectrum is 25% and 21% respectively; 83% and 31% were obtained by liquid auto sampler injection.

Table 12 summarizes the mass spectral analysis of peaks eluting at ± 15 min.

For the 3 mm plate: The mean relative ratios for m/z 111/112 and 125/112 are 25% and 15% at all source temperatures. The corresponding ratios for the NIST reference spectrum for GSM is 23% and 14% respectively; 25% and 16% were obtained by liquid auto sampler injection.

For the 6 mm plate: The mean relative ratios for m/z 111/112 and 125/112 are 25% and 15%. The corresponding ratios for the NIST reference spectrum for GSM is 23% and 14% respectively; 25% and 15% were obtained by liquid auto sampler injection.

Based on the NIST reference mass spectra, and the liquid auto sampler injections, the data indicate that under the purge-and-trap/

GCMS conditions, 2-M-2-B elutes at ± 9 min, 2-MIB elutes at ± 12 min, and GSM elutes at ± 15 min.

Table 13 summarizes the area precision data: For the 3 mm plate: Optimum precision, based on all three target ions, was obtained at 290°C for both 2-M-2-B (4.7%, 4.6% and 5.3% for m/z 95, 107, 108) and GSM (3.4%, 4.3% and 3.1% for m/z 111, 112, 125).

For the 6 mm plate: Optimum precision was obtained at 310°C for 2-M-2-B (12.5%, 1.5% and 1.0% for m/z 95, 107, 108) and at 250°C for GSM (0.7%, 0.6% and 1.0%, for m/z 111, 112, 125).

Mass Spectral Analysis of 2-Methyl-2-Bornene (2-M-2-B)

The reference mass spectral data of 2-M-2-B (CAS Number: 072540-93-3) and 2-methylenebornane (2-MB) (CAS Number:

Drawout Plate (mm)	Source temperature (°C)	t _r (min)	% m/z 111/112	% m/z 125/112
3	230	15.18	22	14
	250	15.18	23	14
	270	15.18	24	15
	290	15.18	25	15
	310	15.18	25	15
	330	15.18	26	16
	350	15.18	27	16
Mean		15.18	25	15
SD		0.00	2	1
RSD%		0.00	6.99	5.44
6	230	15.19	23	14
	250	15.19	23	14
	270	15.19	24	15
	290	15.19	25	15
	310	15.19	26	15
	330	15.19	26	16
	350	15.19	27	16
Mean		15.19	25	15
SD		0.00	2	1
RSD%		0.00	6.33	5.44

Table 12: SIM mass spectral analysis of composite 2-MIB/GSM standard by purge-and-trap: 30 ng/L in MQ.

Plate (mm)	Source (°C)	t _r (min)	^a RSD% m/z 95	^b RSD% m/z 107	^b RSD% m/z 108	t _r min	GSM RSD% m/z 111	GSM RSD% m/z 112	GSM RSD% m/z 125
3	230	9.16	6.33	6.57	5.81	15.18	6.49	18.09	6.56
	250	9.17	23.67	23.60	24.67	15.19	10.96	30.26	11.02
	270	9.18	14.66	14.22	14.89	15.19	4.76	0.96	4.70
	290	9.18	4.68	4.64	5.28	15.19	3.43	4.28	3.10
	310	9.18	10.08	10.52	10.85	15.19	1.94	10.96	1.23
	330	9.18	31.39	19.41	20.35	15.19	4.51	4.64	4.51
	350	9.17	16.68	13.73	14.92	15.19	2.08	6.15	2.25
6	230	9.19	12.37	3.81	12.62	15.19	4.71	4.33	4.20
	250	9.19	4.30	26.84	7.08	15.19	0.72	0.56	1.02
	270	9.19	10.18	15.37	2.57	15.19	0.90	1.01	0.92
	290	9.18	10.89	11.87	12.05	15.19	5.14	4.29	4.37
	310	9.19	12.47	1.45	1.02	15.19	11.75	10.96	10.98
	330	9.20	17.35	32.74	10.98	15.19	3.86	4.24	3.77
	350	9.19	18.40	4.39	7.73	15.19	6.40	6.11	6.55

^a30 ng/L in MQ water

^bpeak at ± 9.2 min

Table 13: Precision of area response by SIM mass spectral analysis of composite 2-MIB/GSM standard^a by purge-and-trap.

2-M-2-B m/z	2-M-2-B Abundance (%)	2-MB m/z	2-MB Abundance %
42	13.00	42	4.60
43	22.60	43	9.40
44	9.00	44	-
49	9.20	49	-
50	-	50	6.60
51	13.70	51	-
52	-	52	6.40
53	18.80	53	13.00
54	7.60	54	4.60
55	19.30	55	11.20
56	12.10	56	-
57	10.30	57	5.70
58	-	58	4.60
63	-	63	5.70
65	15.70	65	9.40
67	28.90	67	15.30
68	8.70	68	6.40
69	29.40	69	15.10
77	26.00	77	14.60
79	98.61	79	26.50
80	13.00	80	5.20
81	10.80	81	11.90
82	9.60	82	8.70
83	-	83	4.60
91	53.41	91	20.30
92	15.70	92	5.90
93	75.87	93	22.80
94	59.21	94	6.80
95	35.60	95	6.80
105	13.40	105	9.10
106	18.40	106	11.20
107	100	107	100
108	20.60	108	15.80
121	24.40	121	19.20
122	8.50	122	-
135	44.40	135	13.20
150	21.50	150	19.90

Table 14: NIST Reference mass spectral analysis for 2-methyl-2-bornene (2-M-2-B) and 2-methylene-bornane (2-MB).

027538-47-2) were obtained from the GCMS Chemstation Software; they were also confirmed by the NIST reference spectra, obtained from NIST [29]. Both compounds have similar molecular weight of 150 and differ in the position of the double bond. However, examination of the mass spectral fragments (Table 14) indicate some differences: beside the similar base peak (m/z 150) for both compounds: the abundances of similar fragments observed in the mass spectra, are significantly greater for 2-M-2-B than for 2-MB to allow unambiguous assignment of either one if present: m/z: 67 (29% vs. 15%), 69 (29% vs. 15%), 79 (99% vs. 27%), 91 (53 vs. 20), 92 (16% vs. 6%), 93 (76% vs. 23%), 94 (59% vs. 7%), 95 (36% vs. 7%) and 135 (44% vs. 13%).

Of note is the base peak of m/z 107 for 2-M-2-B (Figure 6). The relative ratios of m/z 95/107 and 108/107 are 35.65% and 20.60% respectively. The relative abundance of the molecular ion, m/z 150, is 21.50%.

Dehydration of 2-MIB to 2-M-2-B

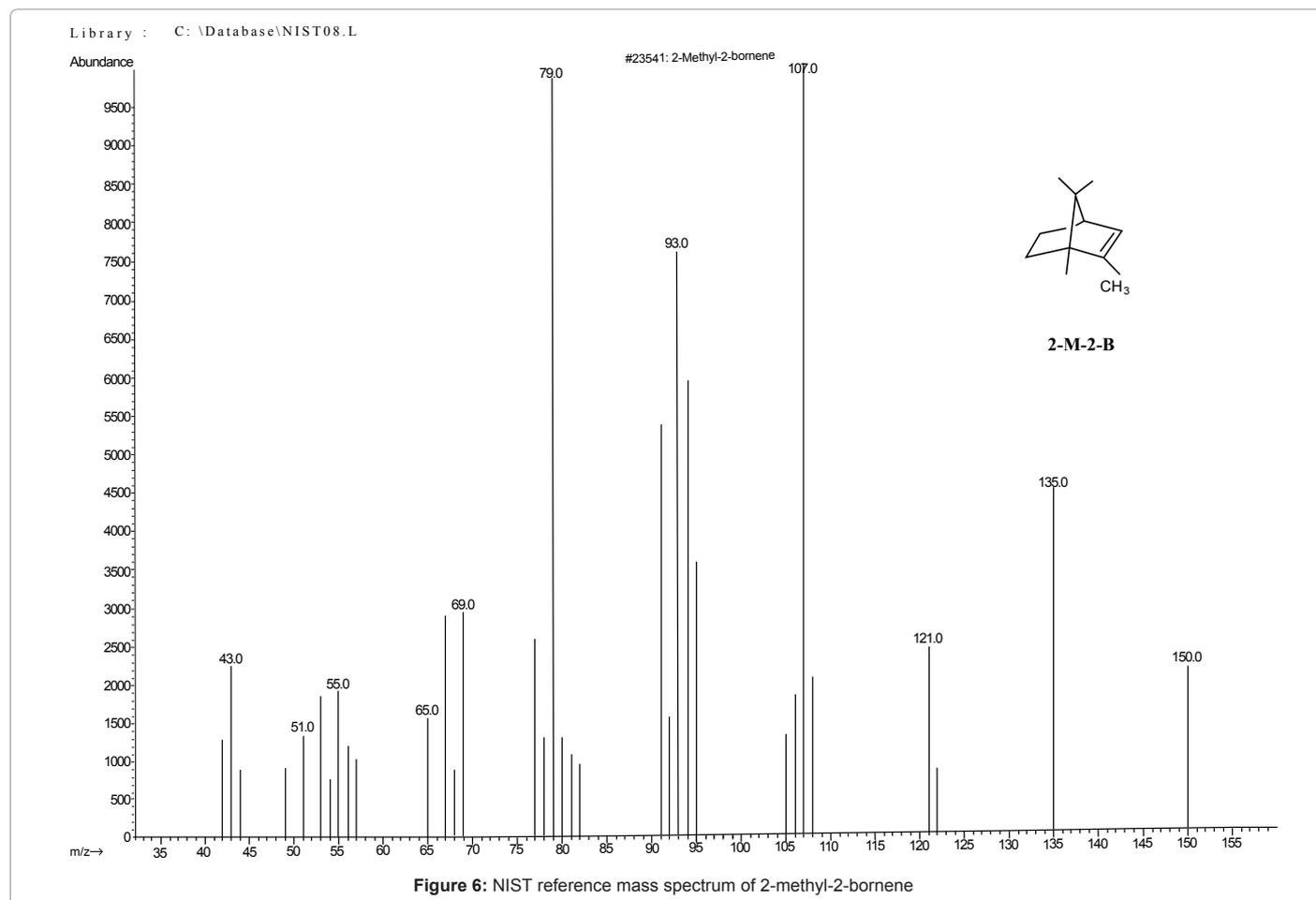
Martin et al. [33] reported off-flavor in the channel catfish due to 2-MIB, and its two dehydration products: 2-methylene-bornane (2-

MB) and 2-methyl-2-bornene (2-M-2-B). Separation was done on a fused silica capillary column, coated with cross-linked methyl silicone; retention times and mass spectrometric analysis was used to confirm identification.

A recent paper [34] reported the biosynthesis of 2-MIB, and a minor amount of the three corresponding metabolites: 2-MB, 2-M-2-B, and 1-methylcamphene. A neutral bond-5 capillary column (5% phenylmethylsilicon) was used for gas chromatographic analysis, with mass spectrometric detection for compound identification; 2-MB and 2-M-2-B were chemically synthesized for this purpose [34].

Under their [34] gas chromatographic conditions, the relative retention times were ± 6.01 , ± 6.42 and ± 8.00 min, for 2-M-2-B, 2-MB, and 2-MIB. The earlier elution of 2-M-2-B and 2-MB is consistent with these relatively non-polar molecules which lack the polar hydroxyl group.

Under the purge-and-trap/GCMS conditions, we noted that 2-MIB elutes at ± 12 min, as confirmed by comparison with the NIST reference



spectrum and our liquid auto sampler injections. We also noted the presence of another peak at ± 9.18 min, with the 30, 100 and 200 ng/L standards in MQ water (Figure 5). Mass spectral analysis, of this peak shows similarity with the NIST reference spectrum for 2-M-2-B: the base peak is m/z 107 and the relative ratios for m/z 95/107, 108/107, 150/107 were on average: 32%, 20%, and 15% fairly similar to that of the NIST reference spectrum: the base peak is m/z 107 and the relative ratios for m/z 95/107, 108/107, 150/107 is 36%, 21%, 20%.

The earlier elution time of this peak, relative to 2-MIB, is also consistent with the earlier finding [34]. Our findings indicate that the peak at 9.18 min is 2-M-2-B, a dehydration product of 2-MIB.

Both dehydration products were reported to be also present in water, and contributed to off-flavor [33]. The standards used in our purge-and-trap were prepared in MQ water. The latter can therefore be excluded as a source of 2-M-2-B. The pH of the MQ water, averaged over 7 d, was 7.4 (SD=0.6; RSD=7.68%), thus excluding any acid environment required for dehydration of alcohols.

The observation that the dehydration product is absent with liquid injections at source temperatures from 230°C up to 350°C indicates that 2-MIB is stable at these temperatures. Dehydration most likely occurs at some stage prior to the molecule entering the ion source when the purge-trap is used as sample concentrator.

A consideration of the various temperatures used in our purge-and-trap method on the Eclipse equipment, indicate the following:

The water sample (25 mL) sample is heated at 80°C during the purge step for 11 min, is desorbed from the Tenax trap at 180°C, passes through the sample mount, at 90°C, the valve oven, at 140°C, into the transfer line, at 110°C, and enters the injection port of the GC, set at 200°C. The oven program runs from 40°, up to a final temperature of 280°C. The MS interface (thermal auxiliary) temperature is set at 230°C, and the ion source at 230°C as default temperature.

The two major variables between the liquid injection and the purge-trap are the following:

1. The water sample is heated to 80°C; for liquid injections, the methanol sample is at ambient temperature
2. The desorption step, where the trap is heated to 180°C, for 3 min, to desorb the trapped analytes.

For results obtained with the 30 ng/L MQ standard by purge-and-trap:

- **For the 3 mm plate:** the observed peak at ± 9.2 min has relative ratios of 33% and 21% for m/z 95/107 and 108/107, comparing fairly well with 36% and 21% observed for 2-M-2-B.
- **For the 6 mm plate:** the observed peak at ± 9.2 min has relative ratios of 29 and 18% for m/z 95/107 and 108/107, comparing fairly well with 36% and 21% observed for 2-M-2-B.

2-MIB is a tertiary alcohol with b.p 207°C. Alcohols are generally

dehydrated by heating in the presence of strong acid, like sulfuric or phosphoric acid, at high temperature. The required range of reaction temperature decreases with increasing substitution of the hydroxyl-containing carbon [35]:

1° alcohol: 170-180°C

2° alcohol: 100-140°C

3° alcohol: 25-80°C

The relative reactivity of alcohols in dehydration reaction is ranked as follows: Methanol < primary < secondary < tertiary [35].

When more than one alkene product is possible, the favored product is usually the thermodynamically most stable alkene [37]. More substituted alkenes are favored over less-substituted ones; *trans*-substituted alkenes are preferred compared to *cis*-substituted ones. Zaitsev's rule [37] states that the double bond would tend to go toward the most highly substituted carbon. For 2-MIB, two alkenes are possible dehydration products: 2-M-2-B and 2-MB (Figure 7). The more stable product is 2-M-2-B.

These results indicate that 2-MIB undergoes dehydration, under the purge-trap conditions, to 2-M-2-B (Figure 7).

The dehydrated molecule, 2-M-2-B, apparently elutes at ± 9.2 min under the gas chromatographic conditions employed. The approximate amount of 2-MIB, which does not undergo dehydration, 2%, elutes at the retention time of 11.99 min, confirmed by the liquid injection of a methanol standard of 2-MIB. The use of the 6 mm plate and the elevated source temperature, up to 350°C does not significantly reduce the dehydration of 2-MIB.

Discussion of our preliminary method

Our current method utilizes the recently launched OI Analytical Eclipse 4660 sample concentrator.

Unlike previous purge-trap methods [11,12], addition of salt to the water sample is not necessary to achieve adequate sensitivity for the application. The use of salt has potential detrimental effects on the entire sample transfer pathway due to salt buildup, blockage, corrosion, or leaks [11].

Optimization of the GC/MS operating conditions involved focusing on injection technique and mass spectrometer acquisition parameters, as with our previous method [2]. A pulsed split injection technique was used to maximize efficient transfer of the target analytes to the GC column. The purge-trap, "narrow bore" liner was found to give improved precision compared to the Supelco liner previously used by us [2]. A low split ratio of 3:1 was used to maximize mass transfer to the detector. The transfer line was directly connected to the carrier

gas line on the split/splitless inlet and insulated to prevent development of a cold spot. The septum purge vent was plugged to prevent further losses.

Target analytes are purged from a 25 mL water sample, at a sample temperature above ambient, and greater than 60°C [2]: 80°C, for 11 min, using helium gas, at a flow rate of 40 mL/min and are adsorbed onto a # 7 Tenax trap. Although the Infrasparg heater rapidly heats the water sample to the desired temperature, we found optimum precision of area responses when a pre-heat time of 1.0 min was used.

The pre-chilling of the water samples down to $\pm 4^\circ\text{C}$, with the Caron temperature circulator, has the added benefit for enhanced target analyte stability in the stored samples prior to analysis. This additional accessory is a significant omission in the earlier reported procedure [2], where the Tekmar-Stratum sample concentrator was used. A dry purge step, of 3 min duration, is included to remove any traces of water that may have accumulated on the Tenax sorbent. The latter step appears to be adequate as virtually no interfering or broad peaks were noted on the resultant gas chromatograms; overall peak shape and symmetry of the taste-odorants were acceptable. Desorption of target analytes from the trap is at a temperature of 180°C, for 3 min. The desorbed analytes enter the GC inlet via a transfer line, set at 110°C.

The "wash" cycle for the purge-and-trap extractor and autosampler, between runs, uses water at ambient temperature for cleaning; hot water (90°C) is used in the Tekmar-Stratum instrument [2].

A conventional Agilent GC-MS system is used for chromatographic separation of the taste-odorants, and subsequent detection or quantitation.

The HP5-MS column, of 30 m length, commonly used for gas chromatography, was used without further variation. The use of a much longer fused silica capillary column (DB 624), of 75 m length, for the similar application, was recently reported [12]. The GC column flow rate of 1.2 mL/min was used; use of 1.0 mL/min did not result in any significant overall improvement in the chromatography, sensitivity or precision of the method. The split ratio of 3:1 was found to be optimum for analyte response. We found significantly improved precision by disabling the septum purge flow (Table 2). A recent report [12] using purge-trap, utilized the splitless mode, with the absence of a transfer line. The oven temperature program is similar to the one recently reported by us [2].

In this method, regarding the mass spectrometer parameters, the EMV mode used is the recently developed Gain Factor setting, compared to our previous method [2], where the Atune voltage and an increment of +400 volts was used. The former allows for better comparison between different voltages used in test methods. Overall precision of responses, as the multiplier ages, is also claimed to be improved [38] with the use of the Gain Factor mode. We also reduced the number of ions used in the SIM mode from 4 to 3 for both taste-odorants, with expected increase in overall sensitivity of the method. Mass fragment m/z 135, for 2-MIB, and m/z 97, for GSM, was omitted. Dwell times were not optimized but were set at 100 msec for all 6 ions. The factory fitted 3 mm drawout plate in the source body, and the default source temperature of 230°C, was initially used, as with our previous procedure [2].

Under the chromatographic conditions of our method, the relative retention times noted were ± 9 min, ± 12 min and ± 15 min. for 2-M-2-B (dehydration product of 2-MIB), 2-MIB and GSM, respectively.

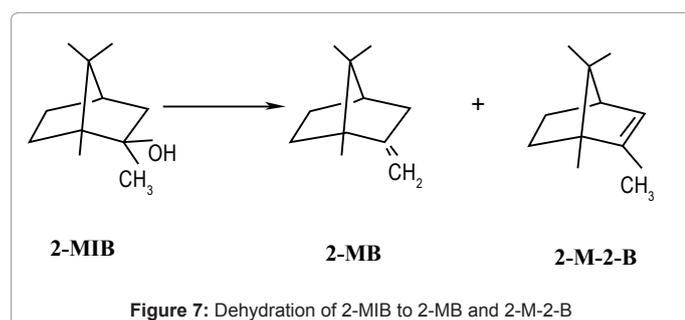


Figure 7: Dehydration of 2-MIB to 2-MB and 2-M-2-B

The method is able to easily detect 2-M-2-B and GSM down to 5 ng/L, with an observed linear analytical range of 5-100 ng/L. Future work will involve confirmation of the limits of quantitation by suitable, established method validation procedures.

The relative area responses of the peaks in the SIM Total Ion Chromatograms for 2-M-2-B and GSM were noted to be in the ratio of approximately 1: 1.7, respectively.

Initial precision results using the standard 3 mm drawout plate, and the default source temperature of 230°C, were as follows:

For the analysis of a 30 ng/L composite standard of 2-MIB and GSM in MQ water, the precision (n = 3 replicates) for the area responses for ions m/z 95, 107 and 108, used for 2-MIB, were 6.33%, 6.57% and 5.81% (RSD), respectively, for the peak eluting at ±9.2 min. The relative ratios for m/z 107/95 and 108/95 averaged 284% (RSD = 0.24%) and 61% (RSD = 0.88%).

The corresponding ratios for m/z 95/107 and 108/107 averaged 35 (RSD = 0.24%) and 22 (RSD = 1.00%).

For ions m/z 111, 112 and 125, used for GSM, the observed RSD was 6.49%, 18.09% and 6.56%, respectively, for the area responses for the peak eluting at ±15.2 min. The relative ratios for m/z 111/112 and 125/112 averaged 22 (RSD = 0.35%) and 14 (RSD = 0.53%).

Implications for Past and Future Purge-and-Trap/GC-MS Analysis of 2-MIB

The recommended temperature for the desorption of adsorbed analytes for the Tenax trap No. 7 is 180°C, for 0.5-4 min duration [39].

Johnsen and Lloyd [10] used traps containing Carboxpack B and Carboxieve SIII for 2-MIB analysis in catfish samples. Trapped 2-MIB was eluted with hexane solvent, presumably at room temperature.

In a later report, Lloyd et al. [3] used a carboxpack/carboxieve trap to trap 2-MIB. Desorbed materials were washed from the traps with hexane.

In both of these early purge-trap/GCMS methods, no information or comment is given on the relative ratios of m/z 95 and 107 for 2-MIB.

The Technical Application Note by OI Analytical [11] reports the use of a Tenax (#7) trap for adsorption of these taste-odorants. Desorption of trapped analytes was done at 180°C for 3 min. Surprisingly, their full scan mass spectrum of a 100 ug/L stock by auto sampler liquid injection shows m/z 107 as the base peak; they even stated that there are literature reports of potential interferences with m/z 107. From the figure 1 supplied, we estimated the relative abundance of m/z 107/95, 108/95 and 150/95 to be 135%, 38% and 29% respectively. The NIST reference spectrum for 2-MIB shows the base peak to be m/z 95; the relative abundance for m/z 107/95, 108/95 and 150/95 is 25, 21 and 7% respectively for the NIST reference spectrum.

From the figure 6 supplied [11], we also estimated the relative abundance of m/z 95/107, 108/107 and 150/107 to be: 74%, 28% and 22% respectively. For the 2-M-2-B NIST reference spectrum, the base peak is m/z 107, and the relative ratios for m/z 95/107, 108/107 and 150/107 are 365%, 21% and 22% respectively.

However, their [11] replicate analysis (n = 9), by purge-and-trap, in SIM mode, of a 5 ng/L composite standard showed that mean area response for ions m/z 95 and 107 for 2-MIB was 1003 and 520 units. It would appear that their results show consistency in the base

peak observed (m/z 107) for liquid auto sampler and purge-and-trap injections, under the instrumental conditions employed.

Based on these data reported [11], the implication is that the dehydration product, 2-M-2-B, rather than 2-MIB, was actually apparently monitored.

The respective retention times for 2-MIB by liquid auto sampler injection, and purge-and-trap, if clearly stated [11] would have assisted in establishing peak identification.

Salemi et al. [12] used a Tenax trap for adsorption of the target analytes. The trapped analytes were desorbed at 250°C for 4 min. Again, no information is given on the relative ratio of ions m/z 95 and 107 for 2-MIB.

We recently reported [2] a validated method for these odorants on the Tekmar Stratum equipment, where it was noted that ion m/z 95 gave optimum accuracy, precision and linearity for 2-MIB. In retrospect, in the SIM mode, the area response ratios were reviewed for m/z 107/95 and 108/95 and was on average 29% and 27%, for composite standards 5, 30 and 100 ng/L in MQ, that is, the abundance for m/z 95 is greater than that of 107 and/or 108 [2]. The NIST reference spectrum (scan mode) for 2-MIB has m/z 95 as the base peak, and the relative ratios for m/z 107/95 and 108/95 is 25% and 21%. The purge-trap parameters involved purging the water sample at 60°C for 20 min; desorption from the Tenax trap was at 180°C, for 4 min [2].

Consideration of the Stratum and Eclipse purge-and-trap parameters, with liquid injection by auto sampler, regarding temperature, indicate the following:

1. A purge sample temperature of 60°C, for 20 min, is used in the Stratum [2], compared to 80°C, for 11 min, in the Eclipse.
2. There is no heating of the methanol standard (e.g., 80°C, used in purge-and-trap) for liquid auto sampler injection.
3. The Tenax trap is heated at 180°C, for 3-4 min on both the Stratum [2] and Eclipse models.

The majority of reported headspace solid phase micro extraction techniques use a sample extraction temperature of 65°C [24-27]. It is noted that three of the reported purge-and-trap methods use ambient temperature for the purge step [3,10,12] and one report uses 80°C [11], without any significant comment (Table 1).

Dehydration of 2-MIB, under purge-and-trap sample extraction, presumably does not occur inside the ionization chamber of the mass spectrometer. The latter is deduced from the observation that for liquid injections of 2-MIB, as a methanol solution, there is no observable dehydration of 2-MIB at all the source temperatures tested, from default 230°C up to the maximum of 350°C, confirmed by absence of the dehydrated product at ± 9.2 min in all the chromatograms.

In an attempt to analyze authentic commercial standards of 2-M-2-B and 2-MB, by GC-MS, to confirm our findings, it was noted that these chemical standards are not commercially available.

Proposed future research

In order to setup an accurate, precise purge-and-trap method for the trace analysis of these taste-odorants, our future GC-MS work on the new Eclipse model, and the Agilent GCMS, to investigate the effect, if any, on the observed ratios of m/z 95 and 107 for 2-MIB, will include the following:

- Effect of purging the water sample at 60°C and lower.
- Top-of-the trap injection of suitable standards onto the Tenax trap, by-passing the 11 min purge step.
- Prior heating of methanol standards of 2-MIB, up to 60°C, followed by liquid auto sampler injection.
- Synthesis of 2-M-B, by the reported synthetic route, from 2-MIB by treatment with thionyl chloride in pyridine [40].

Conclusion

Until further work is done, our preliminary test method development for the assay of taste-odorants in water samples on the Eclipse sample concentrator indicates suitability of the technique for GSM over the analytical range 5-100 ng/L. Based on earlier work by OI Analytical, the instrument has potential for detection of GSM down to 1 ng/L.

Under our purge-and trap conditions used, we have found that 2-MIB dehydrates to 2-methyl-2-bornene (2-M-2-B) during the purge-and-trap cycle conditions used, presumably when the sample is initially purged at 80°C, at all mass spectrometer source temperatures, ranging from the default 230°C, up to the maximum allowed by the instrument configuration, 350°C. Comparison of the 3 and 6 mm drawout plates indicate better area precision is obtained using the 3 mm plate, at source temperature 290°C, for ions *m/z* 95, 107 and 108, characteristic of 2-M-2-B. For GSM, our data indicate better area precision is obtained using the 6 mm drawout plate, at source temperature 250°C. The relative retention times for 2-M-2-B, 2-MIB and GSM were ± 9.2 , ± 12.0 min and 15.2 min, under the gas chromatographic conditions.

We believe that this study will be very useful to current and future analytical scientists, chemists, researchers, etc., using purge-and-trap/GC-MS as an analytical technique for trace analysis of taste-odorants, notably 2-MIB, and GSM, in water.

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