Comparison of Analytical Techniques for Detection of Geosmin and 2-Methylisoborneol in Aqueous Samples

Barry Hurlburt*, Steven W. Lloyd, and **Casey C. Grimm**
USDA-ARS-SRRC, 1100 Robert E. Lee Blvd., New Orleans, LA 70124

Abstract

Geosmin and 2-methylisoborneol are secondary metabolites expressed by a variety of organisms that are responsible for off-flavors in public water supplies, aquaculture, and a host of other important products. Hence, there is continuing research into the causes for their expression and methods to mitigate it, which require sensitive and accurate detection methods. In recent years, several new techniques for collecting and concentrating volatile and semi-volatile compounds have been automated and commercialized, making them available for use in most laboratories. In this study, we compared solid-phase microextraction (SPME) and membrane-assisted solvent extraction (MASE) for the detection of 2-methylisoborneol and geosmin in aqueous samples. SPME is the most sensitive of these techniques with a limit of detection of 25 parts-per-trillion for 2-methylisoborneol and 10 parts-per-trillion for geosmin but with a large relative standard deviation. MASE is less sensitive, but provides a greater level of precision, as well as the ability for multiple injections from the same sample.

Introduction

Ubiquitous in nature, 2-methylisoborneol (2-MIB; 1-R-exo-1,2,7,7-tetramethyl bicyclo-[2-2-1]-heptan-2-ol) and geosmin (trans-1,10-dimethyl-trans-9-decalol) are produced by cyanobacteria in water, actinomycetes in the soil, and fungi and bacteria on every conceivable substrate (1–3). They cause chronic off-flavor problems in aquaculture and hamper industries that are responsible for producing drinking water, cereal, sugar, whiskey, and paper tissue products. Whereas 2-MIB is generally associated with a muddy odor, geosmin has more of a musty or old-book odor. It is, however, nearly impossible to distinguish between them in off-flavor catfish. Humans can detect the presence of 2-MIB or geosmin at levels approaching 10 parts-per-trillion (ppt) in pure water (4) and approximately 0.7 parts-per-billion (ppb) in fish tissue (5). These compounds are non-toxic at concentrations greater than those found in nature. However, they may signal the presence of other dangerous compounds that are also co-produced by the responsible organism (6).

Numerous investigations are underway to understand the reason these compounds are expressed and to mitigate their occurrence. Reliable and sensitive detection methods are needed to support this research and monitor their concentrations in food and water systems.

The advent of solid-phase microextraction technology (SPME) has greatly advanced the analysis of volatile compounds (7). The SPME methodology augments both headspace and purge-and-trap techniques for rapid qualitative and semi-quantitative analyses. The relative low cost, ease of use, and extensive capabilities of SPME have resulted in a wide range of applications in the analysis of foods (e.g., fruits and vegetables) and environmental samples (soil and water) (8,9). Using SPME, the off-flavor odorants 2-MIB and geosmin can be readily detected in water at concentrations approaching the low ppt range (10,11). However, SPME has found limited use for true quantitative work. Multiple factors, including a double equilibrium (sample to headspace, headspace to fiber), slight variations in the matrix (e.g., moisture, inhomogeneity of the sample), and the presence of interfering compounds can result in large variations in the amount of analyte collected from sample to sample. Routine analyses of geosmin and 2-MIB in catfish pond water frequently give inconsistent results (Grimm, unpublished data). Because samples are often collected and prepared at remote field sites, re-analysis of the sample is not possible. Consequently, a more precise analytical method is needed for the routine analyses of these compounds from pond water samples.

An alternative to SPME is membrane-assisted solvent extraction (MASE) (12,13). Developed by Popp and others at the Environmental Research Center at Leipzig-Halle, MASE is a liquid–liquid extraction, which employs low-density polypropylene bags to separate the two liquids. Typically the polypropylene bag is filled with 0.5–1 mL of organic solvent and immersed into an aqueous sample in a 20-mL vial. The analytes traverse the polypropylene membrane and partition between the aqueous and organic phases. Salt can be added to the aqueous phase to enhance the partitioning towards the organic phase. A concentration factor of one to two orders of magnitude can be obtained due to the low amount of organic solvent employed. Increased

*Author to whom correspondence should be addressed: email barry.hurlburt@ars.usda.gov

sensitivity is accomplished by employing large-volume injection, which permits the analyses of analytes at concentrations in the ppb range.

In the work described here, the automated techniques of SPME and MASE are compared in the analysis of aqueous samples containing 2-MIB and geosmin. Although SPME method is inherently more sensitive because all of a given analyte can be collected and transferred to the injection port, MASE employs only a single equilibrium and allows for multiple injections, thus offering the possibility of greater precision.

Experimental

Standards

Geosmin (9a, 10a-decalol; CAS#: 19700-21-1), 2-Methylisoborneol ([1R-exo]-1,2,7,7-tetramethyl - [2,2,1]-bicycloheptan-2-ol; CAS#: 2371-42-8), and decahydro-1-naphthol (DHN; *cis*-Decahydro-1-naphthol; CAS#: 36159-47-4) were obtained from Sigma-Aldrich (St. Louis, MO). Standards of 2-MIB and geosmin at a concentration of 100 ppm in methanol were diluted to 200 ppb in hexane to produce stock solutions. Samples were then prepared in deionized water from the stock solution at concentrations of 0.01, 0.05, 0.1, 0.5, and 1.0 ppb.

Algal samples

Unialgal cultures of *Pseudanabaena* sp., *Oscillatoria splendida*, *Oscillatoria chalybea,* and *Oscillatoria princeps* were grown in modified BG-11 medium (14) with a 12 h light/dark cycle. Approximately 35 µmol m^2/s of light were provided by cool-white fluorescent lighting. 6 mL (SPME) and 16.5 mL (MASE) of the cultures, including the cells, were used in the analysis. *Oscillatoria splendida* produces geosmin, whereas the other three make 2-MIB.

SPME

Three grams of NaCl were placed in a 20-mL vial, and 12 mL of an aqueous sample was added to the vial. The vial was sealed with a twist cap, fitted with a Teflon-lined septum, and placed in a CTC Analytics AG, Combi-PAL autosampler (Leap Technologies, Carrboro, NC) equipped with a 1-cm long divinylbenzene–carboxen–polydimethylsiloxane SPME fiber (Supelco, Bellefonte, PA). Samples were maintained at room temperature until analyzed. The sample was then heated to 65ºC, and the SPME fiber was inserted into the headspace for a 15 min adsorption period while undergoing vigorous agitation. The fiber was withdrawn from the sample and desorbed at 270ºC for 2 min in the injection port of the GC. The fiber was then baked for an additional 4 min in an external heating block to prevent carryover. The injection port was operated in pulsed splitless mode and fitted with a 0.7 mm i.d. injection liner. The head pressure was set to 25 psi of helium for the first minute, and then to a constant flow of 1.1 mL/min to give a velocity of 40 cm/s.

MASE

A magnetic stir bar and 3 g of NaCl were placed in a 20-mL sample vial. Aqueous solutions (16.5 mL) of 2-MIB and geosmin were then added. A polypropylene bag (Gerstal, Baltimore, MD) containing 1 mL of hexane and 1 ng/mL of DHN was immersed into the sample vial. A twist cap with a teflon liner was then used to seal the vial. The sample vial was then stirred for a minimum of 2 h at room temperature prior to analysis. Large-volume injection (LVI) of samples was accomplished using a 100-µL syringe and a pressurize temperature vaporizer (PTV; Gerstel, Germany). The PTV was operated in solvent vent mode at a flow of 100 mL/min with the injection port at 65ºC for hexane and an injection speed of 6 µL/s. The GC run was started upon completion of the sample injection, and the injection port temperature was raised at 10ºC/s to 270ºC and held for 2 min. At a GC run time of 2 min, the inlet was vented with helium (50 mL/min). For pentane and cyclohexane, the inlet was held at 35ºC and 75ºC, respectively, during injection.

GC–MS

All samples were run on an Agilent 5973 MSD equipped with an Agilent 6890 GC and a Combi-PAL autosampler (Gerstel, Baltimore, MD). The original injection port was replaced with a programmable pressure temperature vaporizer enabling LVIs. For MASE samples, an inlet liner packed with glass wool was employed, while for SPME samples a reduced volume inlet liner was used in the PTV inlet. A 30 m, DB-5MS (J&W Scientific, Folsom, CA) capillary column with a 0.25 mm i.d. and a 1.0 µm stationary phase was used. Helium was used as the carrier gas at a flow rate of 1.1 mL/min. The oven was initially held at 60ºC for 1 min, then increased by 10ºC/min to 300ºC and held for 4 min. The mass spectrometer was operated using electrion ionization at 70 eV. Selected ions of the base peaks and molecular ion for 2-MIB (*m/z* 95 and 168), DHN (*m/z* 135 and 154), and geosmin (*m/z* 112 and 182) were monitored alternatively at dwell times of 100 µs each. Quantitation was performed by integrating the base peak area.

Results and Discussion

Precision of MASE technique

The tertiary alcohols, 2-MIB, and geosmin are hydrophobic and have a water–1-octanol partitioning co-efficient in excess of 1:40 (15). The solvent for the MASE/LVI technique for 2-MIB and geosmin should be non-polar with a low boiling point. Solutions of 2-MIB and geosmin in pentane, hexane, and cyclohexane were compared for the optimal precision using LVI. In Table I, recovery values for repeated 90-µL injections of a 5 ppb solution (450 picograms of analyte) show that based upon repeatability,

Table I. RSDs for Five Injections of Different Solvents Using MASE

	BP	2-MIB	Geosmin
Pentane	36C	39.29%	14.58%
Hexane	69C	3.38%	2.15%
Cyclohexane	81C	3.95%	9.55%

hexane or cyclohexane should be used as the solvent. The use of pentane as the solvent gave low recovery but also a relative standard deviation (RSD) of 30–40%. RSDs for the LVI technique is more than 90% for hexane and cyclohexane.

The reconstructed ion chromatograms (Figure 1) are *m/z* 95 and *m/z* 112 for standards of 2-MIB and geosmin, respectively. At a concentration of 1 µg/kg, SPME is clearly more sensitive than the MASE method employed. However, because only a portion of the organic phase (9%) is actually injected, multiple injections are possible with MASE, whereas analysis by SPME is limited to a single injection per sample.

Recovery

In addition to incomplete partitioning into the organic phase, the analytes may also be adsorbed onto the walls of the vial, lost into the small headspace just beneath the cap and onto the polypropylene membrane itself. In order to determine the recovery, a solution of the analytes was prepared in hexane at a concentration of 16.5 ppb. This is the concentration one would obtain with 100% recovery if the analyte were to completely migrate into the 1-mL organic phase. Five individual 1 ppb aqueous samples analyzed by MASE compared to five injections of the 16.5 ppb solution gave recovery values of 81% and 85% for 2-MIB and geosmin, respectively. The total difference between the two values can be solely attributed to the recovery of the analytes.

MASE vs. SPME

Limits of detection of 2-MIB and geosmin on this system (GC–MS using SIM) were determined to be 40 pg and 25 pg of material, respectively. SPME employs a 12-mL sample, and theoretically the entire amount can be collected which should give a measurable signal for a 0.003 ppb solution of 2-MIB. For MASE, a slightly larger sample (16.5 mL) volume is analyzed, but only a small portion of the 1 mL of organic phase is injected (90 µL in this experiment). For an injection of 9% of the organic phase, one would expect to see a measurable signal at a concentration of 0.02 ppb.

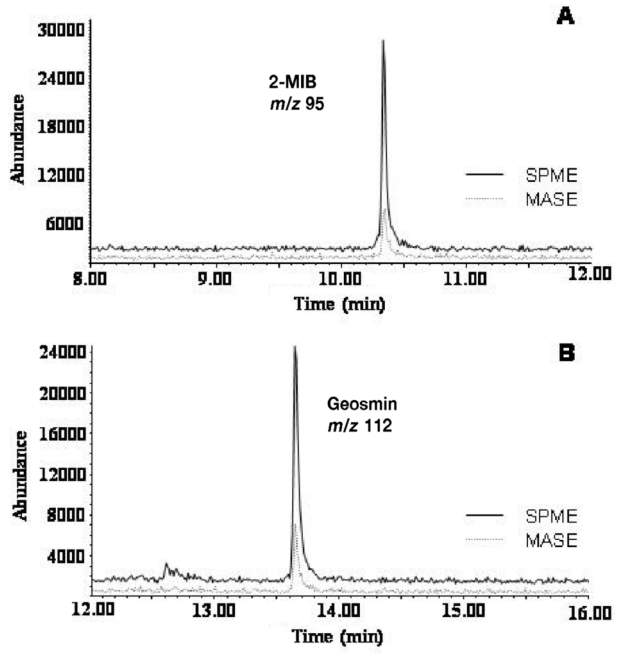

Figure 1. The reconstructed ion chromatograms are shown comparing the analysis of 1 µg/kg standards by SPME and MASE. 2-MIB at *m/z* 95, (A); and geosmin at *m/z* 112, (B).

Table II gives the results for the analyses of a series of concentrations of 2-MIB and geosmin by SPME and MASE. As expected in the 0.005 ppb solution, 2-MIB can be detected using SPME but not by MASE. Using MASE, 2-MIB is not detected until a concentration of 0.05 ppb is injected. RSD are indicative of the reproducibility of the techniques. Published data (10,11) report best efforts and show good reproducibility. However in routine experiments, reproducibility is not quite as good as indicated by the data for SPME in Table II. Wide variation is expected at the low concentration levels of 0.005 ppb and generally improves with concentration. In this particular example the RSDs did not improve and are moderately worse than normally observed.

Algal samples

The two analytical techniques were compared using four algal cultures known to produce off-flavors. These cultures present the off-flavor analytes in a complex matrix which, as opposed to the standards described previously, are representative of real world samples. Averaged values for four analyses, along with RSDs, are presented in Table III. Calculated amounts are in good agreement with each other with the exception of the values for

Table II. RSDs at Specific Concentrations for SPME vs MASE

	SPME		MASE	
2-MIB (ppb)	ng	RSD	ng	RSD
0.005	0.03	30.20%	0.08	ND
0.01	0.06	20.00%	0.16	ND
0.05	0.3	33.50%	0.8	4.40%
0.1	0.6	54.50%	1.6	26.00%
0.5	3	63.90%	8	3.90%
1	6	30.50%	16	6.20%
Geosmin (ppb)				
0.005	0.03	53.30%	0.08	ND
0.01	0.06	54.50%	0.16	ND
0.05	0.3	12.10%	0.8	6.20%
0.1	0.6	56.90%	1.6	8.70%
0.5	3	61.10%	87.40%	
1	6	31.50%	16	2.30%

Table III. SPME vs MASE for Algal Samples

		MASE		SPME	
	off-flavor	ppb	RSD	ppb	RSD
Pseudanabaena sp.	2-MIB	0.32	5.14%	0.39	12.06%
Oscillatoria chalybea	2-MIB	0.42	3.69%	0.43	14.27%
Oscillatoria princeps	2-MIB	0.05	8.09%	0.06	20.93%
Oscillatoria splendida	Geosmin	0.19	6.05%	0.13	38.56%

Pseudanabena, in which case the concentration determined by MASE is lower than the value determined employing SPME (0.32 ppb vs. 0.39 ppb). In general, precision increases with concentration, and the MASE technique gave better precision for these four samples.

Conclusion

The research reported here compares two analytical techniques, SPME and MASE, for the GC–MS analysis of the musty, muddy off-flavors 2-MIB and geosmin. SPME is simpler, less expensive, and more sensitive than the MASE technique. In order to achieve similar detection levels, MASE requires the use of a LVI system. The LVI in turn requires the use of cryofocusing coolants such as liquid nitrogen or CO_2. For consumables, whether operating in manual mode or with an autosampler, SPME only requires the fibers and a reduced volume injection liner. Using more than 100 injections per fiber is not uncommon. With a fiber costing ~$100 each, the SPME sampling cost is ~ $1 per sample (vials, caps, and GC–MS cost are additional). Consumables for MASE include the membranes, magnets, and cryofocusing coolants. Although membranes (~ $5/ea) can be reused, they need to be cleaned between samples. With repeated use, the sealant on the membranes begins to split and only 5–10 analyses are obtained per membrane. SPME is clearly less expensive in manual mode as MASE can not be run without automation. Furthermore, it offers only a single order of magnitude in concentration. However, because of only the single liquid/liquid portioning, it is much more precise by nearly a two-fold factor for both 2-MIB and geosmin. For selected compounds, MASE offers an alternative method to SPME with enhanced repeatability but at a slight increase in cost and sample preparation. Therefore, SPME is the method of choice for applications that require detection of the compounds at the levels where humans can. For applications in which the concentrations are higher and accuracy is important, MASE is the method of choice.

Acknowledgements

We are indebted to Drs. Elaine Champagne and Soheila Maleki for critical reading of the manuscript. This work was supported by intramural CRISs 6435-43440-040-00D and 6435-44000-063-00D from the Agricultural Research Service, U.S. Department of Agriculture. Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the U.S. Department of Agriculture and does not imply approval of the product to the exclusion of others that may be available.

References

1. N.N. Gerber and H. A. Lechevalier. Geosmin, an earthly-smelling substance isolated from actinomycetes. *Appl. Microbiol.* **13:** 935–938 (1965).
2. J. Martin, C. McCoy, W. Greenleaf, and L. Bennett. Analysis of 2-methylisoborneol in water, mud, and channel catfish from commercial culture ponds in Mississippi. *Can. J. Fish. Aqua. Sci.* **44:** 909–912 (1987).
3. N. Wood and V. Snoeyink. 2-Methylisoborneol, improved synthesis and a quantitative gas chromatographic method for trace concentrations producing odor in water. *J. Chromatogr.* **132:** 405–420 (1977).
4. P. Persson. On the odor of 2-methylisoborneol. *Water Res.* **14:** 1113–1118 (1980).
5. P.B. Johnsen and C.A. Kelly. A technique for the quantitative sensory evaluation of farm raised catfish. *J. Sens. Stud.* **4:** 189–199 (1990).
6. C.P Dionigi, T.E. Lawler, P.E. McFarland, and P.B. Johnsen. Evaluation of geosmin and 2-mehtylisoborneol on the histidine dependence of TA98 and TA10 Salmonella typhimurium tester strains. *Water Res.* **27:** 1615–1618 (1993).
7. R. Belardi and J. Pawliszyn. The application of chemically modified fused silica fibers in the extraction of organics from water matrix samples and their rapid transfer to capillary columns. *Wat. Pollut. Res. J. Canada.* **24(1):** 179–91 (1989).
8. J. Pawliszyn, Editor. Applications of Solid-Phase Microextraction. The Royal Society of Chemistry. Cambridge, UK. 407–422 (1999).
9. L.J. Krutz, S.A. Senseman, and A.S. Sciumbato. Solid-phase microextraction for herbicide determination in environmental samples. *J. Chromatogr. A* **999(1-2):** 103–121 (2003).
10. S. Lloyd, J. Lea, P. Zimba, and C. Grimm. Rapid analysis of geosmin and 2-methylisoborneol in water using solid-phase micro-extraction procedures. *Water Res.* **32(7):** 2140–2146 (1998).
11. S. Watson, B. Brownlee, T. Satchwill, and E. Hargesheimer. Quantitative analysis of trace levels of geosmin and 2-MIB in source and drinking water using headspace SPME. *Water Res.* **34:** 2818–2828 (2000).
12. B. Hauser and P. Popp. Membrane-assisted solvent extraction of triazines and other Semi-volatile contaminants directly coupled to large volume injection / GCMS. *J. Sep. Sci.* **24:** 551–560 (2001).
13. B. Hauser, P. Popp, and E. Kleine-Benne,Membrane-assisted solvent extraction of triazines and other semi-volatile contaminants directly coupled to large-volume injection–gas chromatography–mass spectrometric detection *J. Chromatogr. A* **963:** 27–36 (2002).
13. S. Watson, B. Brownlee, T. Satchwill, and E. Hargesheimer. Quantitative analysis of trace levels of geosmin and 2-MIB in source and drinking water using headspace SPME. *Water Res.* **34:** 2818–2828 (2000).
14. D.F. Millie, C.M. Hersch, and C.P. Dionigi. Simazine-induced inhibition in photoacclimated populations of Anabaena circinalis (Cyanophyta). *J. Phycol.* **28:** 19–26 (1992).
15. P. Howgate. Tainting of farm fish by geosmin and 2-methyl-iso-borneol: A review of sensory aspects and uptake/depuration. *Aquaculture* **234:** 155–181 (2004).

Manuscript received August 12, 2008;
Revision received December 4, 2008.