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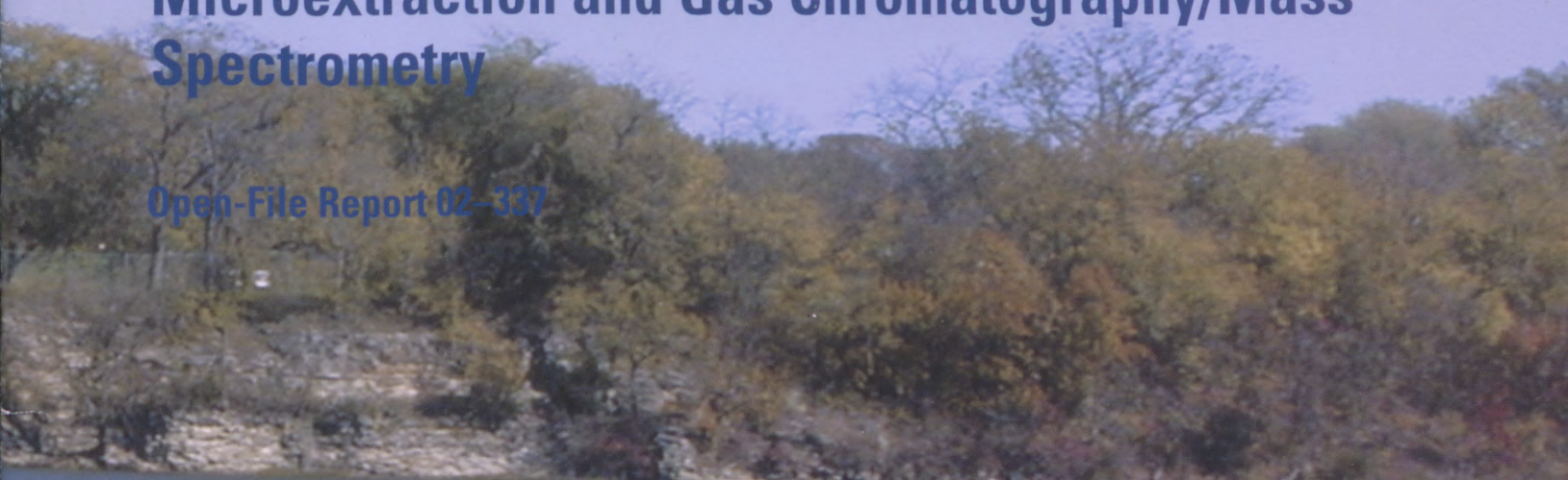
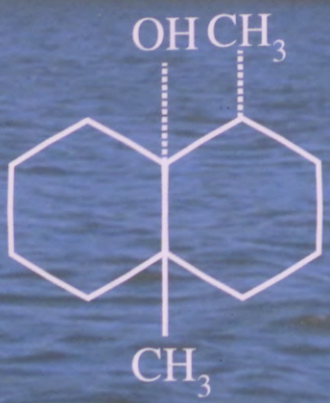
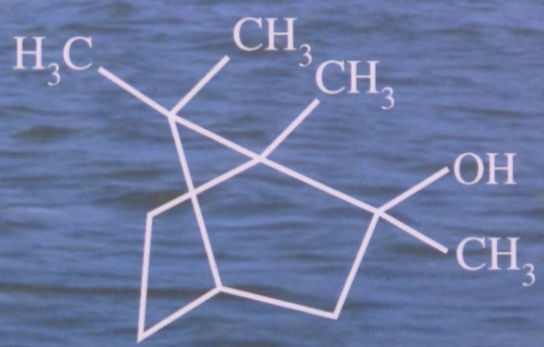
Methods of Analysis and Quality-Assurance Practices by the U.S. Geological Survey Organic Geochemistry Research Group—Determination of Geosmin and Methylisoborneol in Water Using Solid-Phase Microextraction and Gas Chromatography/Mass Spectrometry

Open-File Report 02–337







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By L.R. ZIMMERMAN, A.C. ZIEGLER, and E.M. THURMAN

Open-File Report 02–337

Lawrence, Kansas
2002

U.S. Department of the Interior

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CONTENTS

TABLES

CONVERSION FACTORS, MISCELLANEOUS ABBREVIATIONS AND ACRONYMNS, AND ABBREVIATED WATER-QUALITY UNITS

Conversion Factors

Multiply	By	To obtain
centimeter (cm)	3.937×10^{-1}	inch (in.)
gram (g)	3.527×10^{-2}	ounce, avoirdupois (oz)
inch (in.)	2.54	centimeter (cm)
foot (ft)	0.3048	meter (m)
kilopascal (kPa)	1.450×10^{-1}	pound per square inch (lb/in^2)
liter (L)	3.382×10	ounce (oz)
meter (m)	3.281×10	foot (ft)
microgram (µg)	3.527×10^{-8}	ounce, avoirdupois (oz)
microliter (µL)	3.382×10^{-5}	ounce (oz)
micrometer (µm)	3.937×10^{-5}	inch (in.)
milligram (mg)	3.527×10^{-5}	ounce, avoirdupois (oz)
milliliter (mL)	3.382×10^{-2}	ounce (oz)
millimeter (mm)	3.937×10^{-2}	inch (in.)
nanogram (ng)	3.527×10^{-11}	ounce, avoirdupois (oz)
ounce (oz)	2.957×10^{-2}	liter (L)

Temperature can be converted to degrees Celsius (°C) or degrees Fahrenheit (°F) by the equations:

$$°C = 5/9\ (°F - 32)$$
$$°F = 9/5\ (°C) + 32.$$

Miscellaneous Abbreviations and Acronyms

±	plus or minus
ACS	American Chemical Society
AWWA	American Water Works Association
DVB	polydivinylbenzene
amu	atomic mass unit
GC	gas chromatography
GCG	method code for geosmin and 2-methylisoborneol
GC/MS	gas chromatography/mass spectrometry
IPMP	2-isopropyl-3-methoxypyrazine
m/z	mass to charge
MDL	method detection limit
MIB	2-methylisoborneol
min	minute
MS	mass spectrometry
ms	millisecond
PAC	powdered activated carbon
PDMS	polydimethylsiloxane
PFTBA	perfluorotributylamine
s	second
SIM	selected-ion mode
SPME	solid-phase microextraction
USEPA	U.S. Environmental Protection Agency
USGS	U.S. Geological Survey

Abbreviated Water-Quality Units

L	liter (L)
µg/mL	microgram per milliliter
µL	microliter
mg/mL	milligram per milliliter
mL	milliliter
ng/L	nanogram per liter
ng/µL	nanogram per microliter

Method of Analysis and Quality-Assurance Practices by U.S. Geological Survey Organic Geochemistry Research Group—Determination of Geosmin and Methylisoborneol in Water Using Solid-Phase Microextraction and Gas Chromatography/Mass Spectrometry

By L.R. Zimmerman[1] and A.C. Ziegler[2], *and* E.M. Thurman[2]

Abstract

A method for the determination of two common odor-causing compounds in water, geosmin and 2-methylisoborneol, was modified and verified by the U.S. Geological Survey's Organic Geochemistry Research Group in Lawrence, Kansas. The optimized method involves the extraction of odor-causing compounds from filtered water samples using a divinylbenzene-carboxen-polydimethylsiloxane cross-link coated solid-phase microextraction (SPME) fiber. Detection of the compounds is accomplished using capillary-column gas chromatography/mass spectrometry (GC/MS). Precision and accuracy were demonstrated using reagent-water, surface-water, and ground-water samples.

The mean accuracies as percentages of the true compound concentrations from water samples spiked at 10 and 35 nanograms per liter ranged from 60 to 123 percent for geosmin and from 90 to 96 percent for 2-methylisoborneol. Method detection limits were 1.9 nanograms per liter for geosmin and 2.0 nanograms per liter for 2-methylisoborneol in 45-milliliter samples. Typically, concentrations of 30 and 10 nanograms per liter of geosmin and 2-methylisoborneol, respectively, can be detected by the general public. The calibration range for the method is equivalent to concentrations from 5 to 100 nanograms per liter without dilution. The method is valuable for acquiring information about the production and fate of these odor-causing compounds in water.

INTRODUCTION

Taste-and-odor occurrences have been documented in a number of public-water supply reservoirs (Silvey and others, 1950; Morris and others, 1963; Romano and Safferman, 1963; Silvey, 1966; Kiessling, 1985; Suffet and others, 1996; Bao and others, 1999). Two of the most commonly occurring unpleasant odor-causing compounds in the United States are geosmin and 2-methylisoborneol (MIB). Geosmin is produced primarily by blue-green algae (cyanobacteria) and actinomycete bacteria and imparts an earthy taste and odor at very low concentrations. MIB is produced by certain species of cyanobacteria, primarily *Oscillatoria*. MIB imparts a musty odor and taste to water.

Reservoirs used for public supplies can become eutrophic or hypereutrophic with age and when an overabundance of nitrogen and phosphorus are present (Eynard and others, 2000). Warmwater temperatures and high nutrient levels are conditions conducive to cyanobacteria blooms (Kajino and Sakamoto, 1995; Clarke and others, 1997; Eynard and others, 2000). The cyanobacteria sources of geosmin and MIB can be

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eliminated with conventional water-treatment processes, but the taste and odors remain. Geosmin and MIB can be partially removed by adsorption on powdered activated carbon (PAC) (Muramoto and others, 1995; Chen and others, 1997; Graham and others, 2000). However, the odor threshold for these compounds is very low, and people can detect them in low nanograms-per-liter (ng/L) concentrations in drinking water, typically 30 and 10 ng/L for geosmin and MIB, respectively (Persson, 1980; Korth and others, 1992). Taste-and-odor occurrences may worsen as reservoirs age and fill with silt.

Taste and odor are thought to be largely an aesthetic concern with no health effects. No correlation has been made between the taste-and-odor compounds, geosmin and MIB, and that of cyanobacteria toxins that also may be present and which are toxic at very low concentrations.

Solid-phase microextraction (SPME) is a relatively new and simple method for the analysis of volatile and semivolatile compounds occurring in a wide variety of food, water, and environmental matrices (Belardi and Pawliszyn, 1989; Eisert and Levsen, 1996; Pawliszyn, 1997). SPME relies on the partitioning of organic compounds from a matrix directly into a solid phase.

The traditional method to extract geosmin and MIB from water is closed-loop stripping (Krasner and others, 1983; American Water Works Association (AWWA), 1998). Purge-trap techniques also have been used (AWWA, 1998). SPME has advantages over closed-loop stripping and purge-trap technologies in that a smaller volume of samples is required, the extraction time is faster, the equipment required is less expensive, and SPME uses no solvents.

Analytical methods utilizing SPME in the detection of geosmin and MIB in the low nanograms-per-liter range have been reported previously by Lloyd and Grimm (1999), Mindrup and Shirey (2000), and as a proposed standard method of the AWWA by Eaton and others (1999) and Foster and others (1999). The SPME fiber used consists of a layer of Carboxen™ (a carbon molecular sieve) and a layer of polydivinylbenzene (DVB), each suspended in polydimethylsiloxane (PDMS) (American Public Health Association, 2001). MIB is retained on the Carboxen™, and geosmin, being larger and less volatile, is adsorbed by the DVB polymer coating (Mindrup and Shirey, 2000). An analytical method was optimized for routine use by the U.S. Geological Survey (USGS) Organic Geochemistry Research Group in Lawrence, Kansas, by modifying sample volume, conditioning time, fiber exposure time, and gas chromatography/mass spectrometry (GC/MS) instrument parameters.

The optimized method was validated, and quality-assurance practices were developed for the determination of geosmin and MIB at nanogram-per-liter levels in water samples. The method involves using SPME to isolate the compounds from water samples and GC/MS to identify and quantify these compounds. Quality-assurance practices include evaluation of laboratory blank and spiked samples, instrument performance, and corrective actions. Method detection limits (MDLs) are calculated on the basis of procedures recognized by the U.S. Environmental Protection Agency (USEPA) (1994). Mean recoveries of the compounds from reagent-, surface-, and ground-water samples also are presented.

All water-quality analytical data that are collected by the USGS on a routine basis for release to the public in data reports and databases must be produced using USGS-approved methods by a laboratory that has been approved by USGS (U.S. Geological Survey, 1998). This policy has been established to ensure that USGS data are of known and documented quality, and that the analytical methods used to produce the data are thoroughly tested, documented, and available to the public. The purpose of this report is to document the method and its performance for geosmin and MIB.

The SPME-GC/MS method of analysis described in this report and used by the USGS Organic Geochemistry Research Group in Lawrence, Kansas, has been assigned the method number "O–2137–02" by the USGS Office of Water Quality in Reston, Virginia. The Organic Geochemistry Research Group identifies the SPME-GC/MS method with the analysis code "GCG."

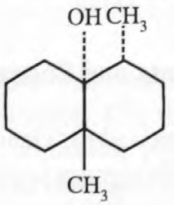
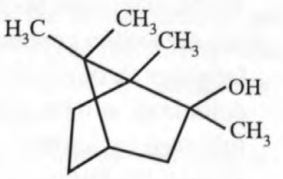
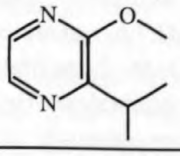
ANALYTICAL METHOD

Scope and Application

The method described in this report is suitable for the determination of nanogram-per-liter concentrations of two odor-causing compounds, geosmin and MIB, in filtered, natural water samples. Chemical structures, parameter codes, and registry numbers are shown in table 1 for each compound determined by the method and for the surrogate standard for these com-

Table 1. Compound name, chemical structure, molecular weight, chemical formula, U.S. Geological Survey (USGS) parameter code, and registry number for compounds determined using method O–2137–02

[amu, atomic mass units; CAS, Chemical Abstract Service; C, carbon; H, hydrogen; N, nitrogen; O, oxygen, --, not applicable]

Compound name	Chemical structure	Molecular weight (amu)	Chemical formula	USGS parameter code	CAS registry number
Geosmin		182.3	$C_{12}H_{22}O$	62719T	23333–91–7
2-methyisoborneol (MIB)		168.3	$C_{11}H_{20}O$	62749T	2371–42–8
2-isopropyl-3-methoxypyrazine (IPMP, surrogate compound)		152.2	$C_8H_{12}N_2O$	--	25773–40–4

pounds, 2-isopropyl-3-methoxypyrazine (IPMP). A parameter code defines sample constituent variables linked to compound analytical results stored in the USGS National Water Information System database. The method is applicable to compounds that are (1) efficiently partitioned from the water phase by SPME and (2) sufficiently volatile and thermally stable for GC. Suspended particulate matter is removed from the samples by filtration, so the method is suitable only for dissolved-phase compounds.

Compounds were selected for determination because of their potential occurrence in public drinking-water supplies. The calibration range for the method is equivalent to concentrations from 5 to 100 ng/L without dilution.

Summary of Method

Water samples are filtered at the collection site using glass-fiber filters (0.7-µm nominal pore diameter) to remove suspended particulate matter. In the laboratory, a surrogate compound (IPMP) is added, and a small volume of sample is removed from the sample container. The sample is conditioned by saturating with salt and heating to partition the compounds to be analyzed into the headspace of the sample container. Then a chemically coated fiber is exposed to the headspace, and the compounds present in the sample are extracted onto the fiber coating. The sample components are desorbed in the hot injection port of a gas chromatograph and separated on a high-resolution, fused-silica capillary column of a GC/MS system.

The compounds are measured and identified under selected-ion mode (SIM). Compounds eluting from the GC column are identified by comparing their measured ions and retention times to reference ions and retention times obtained by the measurement of spiked control samples analyzed under the same conditions used for the water samples. The concentration of each identified compound is measured by relating the MS response of the quantitation ion produced by that compound to the MS response of the quantitation ion produced by the surrogate standard.

Interferences

Organic compounds having identical mass ions and GC retention times to those of the compounds of interest may interfere. The sodium chloride used in conditioning the samples may be a source of interferences, thus it is baked in the laboratory before being used in the method of analysis described in this report.

Apparatus and Instrumentation

- *Analytical balances*—Balance for sodium chloride accurately weighs 10 ± 0.1 g. Balance for surrogate standard preparation accurately weighs 10 ± 0.1 mg.
- *Volumetric glassware*—50- and 250-mL volumes.
- *Autopipettes*—12.5- to 500-µL and 10-mL, fixed- or variable-volume autopipettes with disposable tips (Rainin, or equivalent, Woburn, MA).
- *Laboratory oven*
- *Laboratory dessicator*
- *Water bath*—That will maintain a temperature of 60 to 65 °C.
- *SPME fiber holder*—Supelco part number 57330–U or equivalent (Bellefonte, PA).
- *SPME sampling stand*—Consists of a ring stand with the SPME fiber holder over a water bath.
- *Laboratory timer*
- *SPME inlet guide*—Supelco part number 57356–U or equivalent (Bellefonte, PA).
- *Fused-silica capillary column*—5 percent diphenyl/95 percent dimethyl polysiloxane capillary column (15 m x 0.25 mm inside diameter, 0.25 µm film thickness) (RTX–5MS, or equivalent, Restek Corporation, Bellefonte, PA) coupled to a 5-m guard column.
- *GC/MS benchtop system*—Hewlett Packard (Wilmington, DE), model 5890 series II Plus, or equivalent, connected to a Hewlett Packard, model 5972, or equivalent, MS detector.
- *Recommended GC conditions*—Oven, 60 °C (hold 4 min), then ramp to 270 °C at 10 °C/min, hold for 1 min; injection port, 250 °C; carrier gas, helium; initially a split injection, then splitless injection at 0.75 min.
- *Recommended MS conditions*—Multiplier, 400 over autotune; detector, 275 °C; dwell time, 50 ms; mass ions monitored are listed in table 2 (see section on "Calibration Curve").
- *Data system*—Computer and printer compatible with the GC/MS system used.
- *GC/MS software*—HP DOS ChemStation Software, 1030A version C (Hewlett Packard, Wilmington, DE), is used to acquire and store data and for peak integration.
- *Spreadsheet software*—Microsoft Excel, Microsoft, Inc., Seattle, WA.

Reagents and Consumable Materials

- *Sample vials*, clear borosilicate 60-mL glass vials with septum screw caps; I-Chem, part number S246–0060 or equivalent (New Castle, DE). These vials hold 66 mL when filled to the rim.
- *Reagent water*, generated by purification of tapwater through activated charcoal filtration and deionization with a high-purity, mixed-bed resin, followed by another activated charcoal filtration, and finally distillation in an autostill (Barnstead, or equivalent, Dubuque, IA).
- *SPME fiber assemblies*, 50/30-m DVB/Carboxen on PDMS, 2-cm fiber; Supelco part number 57348–U or equivalent (Bellefonte, PA). Condition the fiber overnight at 270 °C in the inlet of a gas chromatograph.
- *Sodium chloride*, crystalline, American Chemical Society (ACS) grade, ultrapure grade; Fisher Scientific, part number S27–1500 or equivalent (Pittsburgh, PA). Bake overnight at 100 °C and then store in a dessicator to protect the sodium chloride from adsorbing compounds that may interfere with the GC/MS analysis.
- *Weighing pans*, aluminum weighing dishes, flexible for easy pouring; A. Daigger & Company, part number LZ7180A or equivalent (Vernon Hills, IL).
- GC *inlet liner*, narrow bore, Supelco catalog number 2637501 or equivalent (Bellefonte, PA).
- GC *carrier gas*, helium, 99.999 percent.

Standards and Controls

- *Stock standard solutions*—A 100-µg/mL solution of geosmin and MIB in methanol, greater than 99-percent purity; Supelco part number 47525–U (Bellefonte, PA). Prepare a 1.0-mg/mL solution of IPMP (catalog number 297666; Aldrich Chemical Company, Inc., Milwaukee, WI) by accurately

weighing, to the nearest 0.001 g, 50 mg of the pure material in a 50-mL volumetric flask and dilute with methanol. Store at less than 0 °C. This solution is stable for about 24 months.

- *Standard mix for GCG*—A spiking solution of 0.5 ng/µL of geosmin and MIB in methanol. Use 250 µL of the stock standard and dilute with methanol to 50 mL in a volumetric flask. Store at less than 0 °C. This solution is stable for about 24 months.
- *Surrogate standard*—A spiking solution of 0.06 ng/µL of IPMP in methanol. Use the stock standard solution, a 10-µL adjustable pipettor, a 100-mL volumetric flask, and methanol to prepare this solution. To obtain a 22.7-ng/L concentration of surrogate standard in the environmental and control samples, add 25 µL of the surrogate standard spiking solution to the 66-mL sample or standard vial.
- *Calibration and control standards*—Prepare a series of solutions using the GCG standard mix in reagent water at concentrations ranging from 5.0 to 100 ng/L (5.0, 10, 25, 35, 50, and 100 ng/L). Prepare these in 250-mL volumetric flasks and then transfer aliquots to individual 66-mL vials. This yields three calibration and control standards at each concentration. Blank (0 ng/L) calibration and control standards are prepared using unspiked reagent water. The calibration and control standards are processed through the extraction procedure (described in the "Extraction" section).

Sampling Methods

Following USGS protocol, sampling methods capable of collecting water samples that accurately represent the water-quality characteristics of the surface water or ground water at a given time or location are used. Detailed descriptions of sampling methods used by the USGS to obtain surface-water samples are given in Edwards and Glysson (1988) and Ward and Harr (1990). Similar descriptions of sampling methods for obtaining ground-water samples are given in Hardy and others (1989).

Briefly, sample-collection equipment is free of tubing, gaskets, and other components made of nonfluorinated plastic material that might leach interferences into water samples or sorb organic compounds from the water. The water samples from each site are composited in a single container and filtered through a 0.7-µm glass-fiber filter using a peristaltic pump (Sandstrom, 1995). Filters are leached with about 200 mL of sample prior to filtration of the sample. The filtrate for analysis is collected in baked 4-oz amber glass bottles with Teflon-lined lids. Samples are chilled immediately and shipped to the laboratory via an overnight carrier. At the laboratory, samples are logged in, assigned identification numbers, and stored at 4 °C for up to 3 days from time of sample collection before extraction.

Extraction

- *Extraction setup*—An extraction set consists of as many as six samples. In addition to the samples, each extraction set has at least one laboratory sample, a laboratory blank control, a high-concentration spiked control, and a low-concentration spiked control. All the vials in the extraction set are processed identically.
- *Sample preparation*—Environmental samples and control samples are prepared in 66-mL vials filled to the rim. Should a sample contain less than 66 mL, reagent water is added to bring the volume to the required 66 mL. Any volume added is recorded.
- *Spiking of surrogate standard*—Spike 25 µL of surrogate standard (0.06 ng/µL IPMP in methanol) into each vial. All environmental samples, the replicate sample, and control samples then are capped and shaken by hand to assure that the surrogate standard is well mixed.
- *Removal of excess liquid*—Remove 21 mL of water from each environmental and control sample using a pipette with disposable pipette tips. This allows a space for sodium chloride to be added and for the SPME to be performed in the headspace. Forty-five mL of sample will remain in the vial to be extracted.
- *Conditioning of sample*—Add 13.5 g of sodium chloride to each environmental or control sample and vigorously shake by hand to get the sodium chloride into solution. Heat samples using the water bath of the SPME sampling stand to 60 to 65 °C for 35 min. One sample or control is conditioned at 60 to 65 °C for 35 min, while the sample or control before it is extracted at 60 to 65 °C for 35 min. The consistency of the conditioning (temperature, time, and headspace volume) for all samples in an extraction set is imperative.

- *Extraction*—Move the vial to be extracted underneath the SPME syringe support of the SPME sampling stand. Insert the septum-piercing needle of the fiber assembly through the septum of the vial to a depth of 2 in. Expose the SPME fiber to the headspace. Extract for 35 min at 60 to 65 °C. Retract the SPME fiber back into the fiber assembly and immediately proceed to the desorbtion procedure.

Desorbtion

- *Insert the SPME fiber into the injection port*—Using the SPME inlet guide, insert the septum-piercing needle of the fiber assembly through the septum of the gas chromatograph's heated (250 °C) injection port. Use the fiber holder to adjust the fiber to a depth of 3 in. in the injection port. The depth of the fiber coincides with the hottest portion of the injection port and may be different on gas chromatographs from other manufacturers.
- *Expose the SPME fiber in the injection port*—Expose the fiber to the injection port by adjusting the fiber holder. Immediately start the gas chromatograph and leave the fiber exposed in the inlet for 10 min. The 10-min exposure time will regenerate the fiber, and thus it can be used for multiple extractions.

Calibration Curve

- Initial calibration curves are prepared using freshly prepared calibration standards that are extracted using the same procedure as samples (described previously).
- Samples are extracted using the same SPME fiber. This fiber will have had a blank desorbtion (desorbtion without an extraction procedure) and is known, from previous control samples, to be producing adequate extraction recoveries. Damaged SPME fibers can be detected by a visual inspection for areas missing the DVB/Carboxen coating.
- Data are acquired from a GC/MS that meets all performance criteria using the same procedure and method as samples.
- Calculate the relative retention time (RRT_c) for geosmin and MIB in the calibration solution or in a sample as follows:

$$RRT_c = \frac{RT_c}{RT_s}, \quad (1)$$

where RT_c = uncorrected retention time of the quantitation ion of the selected compound or surrogate compound, in minutes, and

RT_s = uncorrected retention time of the quantitation ion of the surrogate standard (IPMP), in minutes.

See table 2 for an example of retention times, relative retention times, quantitation ions, and qualification ions.

- Initial calibration data are entered into a computer spreadsheet, and ratios are calculated for each quantitation ion relative to the surrogate standard (IPMP). Graphs are made from the GC/MS data by plotting the IPMP ratios of a single ion on the x axis and the concentrations of the calibration standards used on the y axis. The spreadsheet determines a trend line for the data points using a linear curve fit. The equation of the trend line and the correlation coefficient (r^2) value appear on each compound's graph.
- Initial calibration data are acceptable if the correlation coefficient (r^2) value for all curves is greater than or equal to 0.99 for each compound.
- Subsequent daily-response factors calculated for the compounds need to agree within ± 20 percent of the mean response factor for the compounds analyzed. A response factor is equal to the area of the quantitation ion for the selected compound or surrogate divided by the area of the quantitation ion for the surrogate standard.

Evaluation of Mass Spectrometer Performance

Mass spectrometer performance is evaluated by assessing isotopic ratios, contamination, electron multiplier sensitivity, instrument response, and peak shape.

- Tune the mass spectrometer before each GC/MS sample set using the procedure and software supplied by the manufacturer. Parameters in the tuning software are set to give ± 0.15-amu resolution at masses 69, 219, and 502 in the spectrum of perfluorotributylamine (PFTBA). With the resolution of the 69 ion at 100-percent abundance, the mass 219 ion should be 35 ± 20 percent, and the mass 502 ion should be more than 3 percent relative

Table 2. Retention times, relative retention times, quantitation ions, and qualification ions for geosmin, 2-methylisoborneol, and surrogate standard analyzed using gas chromatography/mass spectrometry

[min, minute; m/z, mass to charge]

Compound	Retention time (min)	Relative retention time (dimensionless)	Quantitation ion (m/z)	Qualification ion(s) (m/z)
Odor-causing compounds (in order of increasing retention time)				
2-methylisoborneol (MIB)	6.180	1.293	108	95, 107, 135
Geosmin	9.390	1.964	112	97, 125, 149
Surrogate standard				
2-isopropyl-3-methoxypyrazine (IPMP)	4.780	1.000	137	152, 124

abundance; however, the relative abundances may vary depending on the mass spectrometer used. Check mass assignments to ensure accuracy to ± 0.15 amu and that mass peak widths measured at one-half the peak height range from about 0.50 to 0.60 amu.

- Also, during the tuning of the mass spectrometer, check the mass spectrometer for the presence of excessive water and air, which indicate leaks in the vacuum. If detected, locate and fix leaks.
- Initially adjust the electron multiplier of the mass spectrometer to ensure that the established reporting level for each selected compound can be achieved. This is usually at 1,000,000 abundance response of the 69 ion.

Evaluation of Gas Chromatograph Performance

- If peak shape is poor or if compounds fail to meet the calibration criteria, perform maintenance on the capillary column to bring the instrument into compliance. Removing approximately 0.5 m from the head of the guard column often achieves adequate peak shapes.

Calculation and Reporting of Results

Qualitative Identification

- The expected retention times (RT) of the geosmin and MIB peaks need to be within ± 6 s of the expected retention time on the basis of the RRT_c obtained from the surrogate-standard analysis. Calculate the expected retention time (RT) as follows:

$$RT = (RRT_c)(RT_s), \quad (2)$$

where RT = expected retention time of the selected compound, in minutes;
RRT_c = relative retention time of the selected compound, dimensionless; and
RT_s = uncorrected retention time of the surrogate standard, in minutes.

- Mass-spectral verification for each selected compound is done by comparing the relative abundance values of the quantification and qualification ions to the same values obtained from the control standard samples. The relative ratios of the ions need to be within ± 20 percent of the relative ratios obtained in the absence of any obvious interferences.

Quantitation

Calculate the dilution factor to correct for the volume of sample processed as follows:

$$DF = \left(\frac{66}{66 - V_a}\right), \quad (3)$$

where DF = dilution factor; and
V_a = volume added = milliliters of distilled water added to a sample that contains less than 66 mL.

The dilution factor is incorporated into the calculation for determining final concentrations of samples.

- If a selected odor-causing compound has passed the aforementioned qualitative identification criteria, calculate the concentration in the sample as follows:

$$C = \left(\left(\frac{A_c}{A_i}\right)(m) + y\right)(DF), \quad (4)$$

where C = concentration of the selected compound in the sample, in nanograms per liter;
A_c = area of the quantitation ion of the selected compound identified;
A_i = area of the quantitation ion of the surrogate standard;
m = slope of the trend line in the linear curve fit;
y = y intercept of the trend line in the linear curve fit; and
DF = dilution factor as calculated in equation 3.

Reporting of Results

Geosmin and MIB are reported in concentrations ranging from 5 to 100 ng/L. If a concentration is greater than 100 ng/L, the sample is reextracted with a 1:10 dilution (sample:reagent water) and reanalyzed for those compounds that were greater than 100 ng/L.

METHOD PERFORMANCE

A reagent-water sample, a surface-water sample collected from Lake Olathe, Olathe, Kansas, and a ground-water sample collected from a 27-ft deep well near Halstead, Kansas, were used to test the method performance. Aliquots of each sample were fortified with either 10 or 35 ng/L of GCG standard mix. Then they were split into seven 66-mL samples at each concentration (10 and 35 ng/L). In addition, unfortified samples of reagent, surface, and ground water were extracted and analyzed to determine background concentrations of geosmin and MIB. All samples were analyzed in one laboratory (the USGS Organic Geochemistry Research Group in Lawrence, Kansas) using one GC/MS system. Each sample set was extracted and analyzed on different days from March through May 2002, so comparison of different matrices and concentrations included bias from day-to-day variation. Different SPME fibers and, therefore, different standard curves were used. Accuracy and precision data from the analyses are listed in tables 3, 4, and 5.

Corrections for background concentrations—Neither the surface- nor ground-water sample required correction for background concentrations of geosmin or MIB. The reagent-water sample also had no detections of geosmin or MIB.

Method detection limits (MDLs)—An MDL is defined as the minimum concentration of a substance that can be identified, measured, and reported with 99-percent confidence that the compound concentration is greater than zero. MDLs were determined according to procedures outlined by the USEPA (U.S. Environmental Protection Agency, 1994) using fortified reagent water. Reagent water was fortified with 5.0 ng/L of primary fortification standard and split into seven 66-mL samples. These were extracted and analyzed to determine MDLs (table 6). Each sample was analyzed on different days during March through May 2002, so day-to-day variation is included in the results.

The MDL was calculated using the following equation:

$$MDL = (S)(t_{(n-1,\,1-\alpha=0.99)}), \quad (5)$$

where S = standard deviation of replicate analysis, in nanograms per liter, at the fortified concentration;
$t_{(n-1,\,1-\alpha=0.99)}$ = Student's t-value for the 99-percent confidence level with n-1 degrees of freedom (U.S. Environmental Protection Agency, 1994); and
n = number of replicate analyses.

The calculated MDLs were 1.9 ng/L for geosmin and 2.0 ng/L for MIB (table 6). According to the USEPA (1994) procedure, the fortified concentrations should be no more than five times the MDL. The fortified concentrations were within five times the MDL.

Mean accuracy—Mean accuracy in reagent-, surface-, and ground-water samples was determined by comparing the mean observed concentration (see "Quantitation" section) from seven replicate samples to the spiked concentration. Mean accuracy as a percentage of the true concentration was nearly equal (90 to 96 percent) at both concentrations, for both compounds, and for all three matrixes with two exceptions. The first exception was the ground-water samples fortified with 10 ng/L geosmin. The mean accuracy was 123 percent (table 5). The other exception was surface-water samples fortified at 35 ng/L geosmin. The mean accuracy for those samples was only 60 percent (table 4). The mean accuracies for each compound spiked at the concentrations shown in tables 3, 4, and 5

Table 3. Accuracy and precision data from seven determinations of geosmin and 2-methylisoborneol in a fortified reagent-water sample

[ng/L, nanograms per liter]

Replicate sample number	Concentration in samples spiked at 10 ng/L		Concentration in samples spiked at 35 ng/L	
	Geosmin (ng/L)	2-methylisoborneol (ng/L)	Geosmin (ng/L)	2-methylisoborneol (ng/L)
1	9.6	10.9	37.6	31.1
2	7.2	10.1	32.9	33.8
3	8.4	10.1	27.9	26.8
4	10.0	10.2	29.9	29.3
5	9.1	9.5	29.2	31.5
6	9.2	12.6	39.6	30.3
7	9.4	8.1	35.4	39.0
Mean observed concentration (ng/L)	9.0	10.2	33.2	31.7
Standard deviation (ng/L)	.94	1.38	4.48	3.86
Relative standard deviation (percent)	11	13	13	12
Mean accuracy (percentage of true concentration)	90	102	95	91

Table 4. Accuracy and precision data from seven determinations of geosmin and 2-methylisoborneol in a fortified surface-water sample

Replicate sample number	Concentration in samples spiked at 10 ng/L		Concentration in samples spiked at 35 ng/L	
	Geosmin (ng/L)	2-methylisoborneol (ng/L)	Geosmin (ng/L)	2-methylisoborneol (ng/L)
1	10.8	10.9	27.0	30.2
2	10.5	9.0	29.7	33.5
3	9.6	11.8	21.3	33.1
4	9.5	9.1	17.8	34.4
5	8.0	9.1	16.7	34.4
6	6.9	8.8	13.7	31.6
7	7.6	8.2	19.8	36.9
Mean observed concentration (ng/L)	9.0	9.6	20.9	33.5
Standard deviation (ng/L)	1.49	1.28	5.70	2.16
Relative standard deviation (percent)	17	13	27	6
Mean accuracy (percentage of true concentration)	90	96	60	96

Table 5. Accuracy and precision data from seven determinations of geosmin and 2-methylisoborneol in a fortified ground-water sample

Replicate sample number	Concentration in samples spiked at 10 ng/L		Concentration in samples spiked at 35 ng/L	
	Geosmin (ng/L)	2-methylisoborneol (ng/L)	Geosmin (ng/L)	2-methylisoborneol (ng/L)
1	13.1	10.7	30.1	30.3
2	11.1	8.6	31.9	30.0
3	14.9	10.5	30.6	37.4
4	12.0	9.3	29.1	29.4
5	10.7	8.2	32.1	30.0
6	10.1	8.8	34.8	33.3
7	14.1	10.2	35.9	35.0
Mean observed concentration (ng/L)	12.3	9.5	32.1	32.2
Standard deviation (ng/L)	1.79	1.02	2.48	3.08
Relative standard deviation (percent)	15	11	8	10
Mean accuracy (percentage of true concentration)	123	95	92	92

Table 6. Method detection limits calculated for 5.0-nanograms-per-liter concentration in reagent water

Replicate sample number	Concentration, in nanograms per liter	
	Geosmin	2-methylisoborneol
1	5.49	6.35
2	4.20	5.94
3	4.77	5.97
4	4.06	5.29
5	5.70	5.65
6	4.46	6.81
7	4.94	6.96
Mean observed concentration (ng/L)	4.80	6.14
Mean standard deviation (ng/L)	.62	.60
MDL (ng/L)	1.96	1.90

were averaged to calculate the mean recovery for the three matrixes. The mean recovery for geosmin for all three matrixes was 93 percent with a standard deviation of 20 percent. The mean recovery for MIB for all three matrixes was also 93 percent but with a standard deviation of less than 3 percent.

QUALITY-CONTROL DATA

Quality-control data are produced to quantitatively check the measurement process for environmental samples. The types of quality-control data collected include results of the analysis of duplicate samples, laboratory blank samples, and spiked control samples of differing concentrations.

Duplicate Samples

Each extraction set of as many as six environmental samples contains a minimum of one duplicate sample. The samples are laboratory duplicates analyzed concurrently and reanalyzed if agreement of the calculated concentrations for any compound are not within 20 percent, as determined by the relative percentage difference or 5 ng/L, whichever value is greater.

$$RPD = \left| \frac{X_1 - X_2}{\overline{X}} \right| x100, \qquad (6)$$

where RPD = relative percentage difference;
$|X_1 - X_2|$ = absolute value of the difference between the two values; and
$\overline{X}$ = mean of the two values.

Laboratory Blank Samples

Laboratory blank samples are used to demonstrate that laboratory equipment or instruments are cleaned adequately and that no contamination is contributed by the laboratory procedures. A laboratory blank sample consists of reagent water that is processed exactly like environmental samples. If either geosmin or MIB are detected at any concentration greater than the MDL in the laboratory blank sample, the source of the problem is determined and corrected. Samples analyzed in that extraction set then are reevaluated for contamination.

Calibration Verification

Spiked control samples with low and high compound concentrations are used to verify the calibration curve being used for quantification. The recoveries are determined. A new calibration curve is prepared if the recovery is outside the control limits for two consecutive extraction sets. Control limits are initially set at ± 20 percent until an adequate number of control samples have been analyzed to calculate a relevant standard deviation. Control warning limits are set at ± 1.5 standard deviations from the mean and the control limits at ± 2 standard deviations from the mean.

Surrogate Recovery

Recovery of the surrogate, IPMP, is measured by the area counts produced for each sample, including all control samples. Control charts for IPMP recovery are constructed using the mean; the warning limits are set at ± 1.5 standard deviations from the mean and the control limits at ± 2 standard deviations from the mean. The control charts are constructed using all previous sample IPMP recoveries. A sample is reextracted and reanalyzed on the GC/MS if the recovery is outside the control limits. In addition, the sample is analyzed without the addition of IPMP to verify that IPMP is not present in the sample.

CONCLUSIONS

This report presents a method of analysis, method validation, and quality-assurance practices for the determination of the odor-causing compounds geosmin and MIB in natural water samples. From the data presented in this report, SPME with GC/MS detection is shown to be a sensitive and reliable method for the determination of nanogram-per-liter concentrations. Precision and accuracy were demonstrated. Method detection limits were 1.9 ng/L for geosmin and 2.0 ng/L for MIB, which are less than the concentrations typically detected by people. The mean recovery for geosmin for all three matrixes was 93 percent with a standard deviation of 20 percent. The mean recovery for MIB for all three matrixes was also 93 percent but with a standard deviation of less than 3 percent. Information about the production and fate of geosmin and MIB in water can be acquired from the analysis of surface- and ground-water samples.

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