THE STABILITY OF GEOSMIN AND MIB
AND THEIR DEUTERATED ANALOOGUES
IN SURFACE WATERS AND ORGANIC
SOLVENTS

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ABSTRACT

Deuterated geosmin [trans-1,10-[2H3]-dimethyl-9α-decalol] and deuterated methylisoborneol [2-exo-hydroxy-2-[2H3]-methylbornane] were synthesised and evaluated as alternatives to 1-chloroalkanes as internal standards in the determination of geosmin and MIB in surface waters. Geosmin and MIB were stored as dilute solutions in methanol, ethanol, acetone, hexane or carbon disulfide for >6 months without deterioration. Dichloromethane caused substantial decomposition of MIB. When added at the time of sampling, the labelled standards compensated for losses of analyte by physical, chemical and biological processes during sample storage. The labelled compounds were effective internal standards for the determination of other odorous metabolites such as the carbonyl compounds β-cyclocitral, β-ionone, geranylacetone and 6-methylhept-5-en-2-one.

KEY WORDS

geosmin, 2-methylisoborneol, deuterium labelling, analysis, internal standards.

INTRODUCTION

The two compounds most commonly implicated in imparting an earth/musty taste and odour to drinking water are geosmin and 2-methylisoborneol (MIB). They are of particular concern to water managers as they are resistant to normal water treatment processes and can be perceived by the general public at very low levels, typically 10 and 30 ng/l for MIB and geosmin respectively (Tyler et al., 1978; Yasuhara and Fuwa, 1979; Persson, 1980; Bartels et al., 1989). To avoid consumer complaints water authorities generally monitor their source water supply and implement a management strategy while the concentration of either or both of these compounds is still low enough to make an early treatment option viable. To determine these concentrations a sensitive and accurate analytical procedure is required.

Several analytical techniques are now available for the analysis of the two compounds. Our laboratory quantifies geosmin and MIB using a salted closed loop stripping analysis procedure (CLSA) based on that of Hwang et al. (1984) and described in our previous paper (Korth et al., 1991). Although most procedures utilize gas chromatography-mass spectrometry (GC-MS) or GC-flame ionization detector (FID) to quantify the analyte, they differ substantially with respect to sample extraction and concentration.

For accurate quantification the use of an appropriate internal standard is necessary and the diversity of internal standards used to date indicates the difficulty in finding the "ideal internal standard" (Johnsen and Jui Chang, 1987; Hwang et al., 1984). Other workers (Yasuhara and Fuwa, 1979; Tabacheck and Yurkowski,
1976; Dupuy et al., 1987;) resort to the slower standard addition technique. Most laboratories currently use a series of linear 1-chloroalkanes with the CLSA procedure as the best compromise. However, chloroalkanes have a number of disadvantages. They strip at a faster rate than MIB and geosmin, are less sensitive to changes in CLSA parameters than MIB and geosmin (Hwang et al., 1984), require frequent calibration and do not compensate for losses of analyte during sample storage. To overcome these problems deuterated analogues of geosmin and MIB were synthesized. The ring-junction methyl group of geosmin was labelled with three deuterium atoms and the methyl group at C2 in MIB.

Deuterium labelled internal standards have been shown to offer many advantages compared with conventional standards for the determination of MIB and geosmin in surface water (Korth et al., 1991). The identical physical and chemical behaviour of MIB and geosmin to their labelled analogues make the deuterated compounds ideal internal standards when using CLSA-GC-MSD. Variations in CLSA parameters such as stripping time, bath temperature, size and shape of stripping bottle, stripping with or without salt, air flow through the carbon filter, sparging rate or air leaks have no effect on the accuracy of the determination.

The insensitivity to changes in CLSA parameters results in a great deal of time saving because unknowns do not have to be analysed with and without spikes and frequent calibration is not necessary for accurate determinations. If the concentration is sufficiently high the stripping time can be reduced to half an hour or less without affecting the result or having to recalibrate the system. A busy laboratory could easily triple its output, with respect to the analyses of these two compounds, without sacrificing accuracy.

In the present study we planned to measure the stability of standard solutions of geosmin and MIB in various organic solvents and to explore the validity of using (±)-geosmin-d3 (added at the time of sampling) to quantify (-)-geosmin present in river water, with the analysis being performed after varying extents of biodegradation. The suitability of (±)-geosmin-d3 and (-)-MIB-d3 as internal standards for the quantification of other odorous algal metabolites was also investigated.

**MATERIALS AND METHODS**

Synthetic (±) geosmin, MIB, synthetic (±) geosmin-d3, MIB-d3, 1-chlorooctane, 1-chlorodecane, 1-chlorododecane, carbon disulphide, acetone, and dichloromethane were obtained as previously described (Korth et al., 1991) and 6-methylhept-5-en-2-one, geranyl acetone, β-ionone and β-cyclocitral were generously donated by Prof. F. Jüttner.

The CLSA equipment and CLSA-GC-mass sensing detector (MSD) technique used for sample extraction and analysis was as previously described (Korth et al., 1991). GC-FID conditions and equipment used were: GC, Varian 3400; detector, FID (attenuation 4×10⁻¹¹); column, Alltech ECONO-CAP SE 30, 0.25 mm ID, 0.25 μm film; temperature program, 25°C for 1.5 min., then to 250°C at 10°C/min., hold for 5 min.; splitter off 1.5 min.; split flow 50 ml/min.; carrier gas, helium; injector, 260°C; detector, 260°C. Chiral GC was performed with a 50 m J&W Cyclodex-B column with either hydrogen (FID) or helium (MSD) as carrier gas. Temperature program was 30°C hold for 1.5 min. with splitter off, then 30°C/min. with splitter on up to 127°C, hold at 127°C for 38 min., then 30°C/min. up to 230°C then held for 5 min. Ions monitored and the quantifying ions, in brackets, for the four additional odour compounds were as follows: 6-methylhept-5-en-2-one 43, (108), 111, 126; β-ionone 135, (177), 192; β-cyclocitral 109, 123, (137), 152; geranyl acetone 43, 125, 136, (151).

**Storage of MIB and geosmin in organic solvents**

A stock solution of MIB (460 ng/μl), geosmin (450.6 ng/μl), 1-chlorooctane (619.3 ng/μl), 1-chlorodecane (426 ng/μl), 1-chlorododecane (587.3 ng/μl), n-tridecane (446.9 ng/μl) and n-pentadecane (448.9 ng/μl) was prepared in acetone. An aliquot (50 μl) of this solution was added to each of six 4 ml amber screw cap vials containing 2 ml of acetone, dichloromethane, ethanol, methanol, hexane and carbon disulphide respectively. Each solution was mixed thoroughly and analysed immediately. The solutions were split into 2 sets of
duplicate aliquots (1 ml) and stored. The 1st set was stored at -15°C in the dark and the 2nd set at room
temperature (22°C) under normal light conditions. All solutions were analysed at various time intervals (GC-
FID) and the area of each compound was normalised relative to the area of n-tridecane.

Chemical degradation of MIB

MIB (180 mg) was added to CH₂Cl₂ (1 ml) in a 4 ml amber screw cap vial. An aliquot (1 drop) of this
solution was transferred to another vial containing CH₂Cl₂ (2 ml) and analysed by GC-FID. To the initial
solution 1N HCl(aq) (1 ml) was added and the vial placed in a wrist action shaker (48 hrs.). After 27 h a
further 0.9 ml of 10M HCl was added to increase the HCl concentration to 5M. The degradation was
monitored as follows: at various time intervals aliquots (1 drop) were added to CH₂Cl₂ (2 ml) and 5%
NaHCO₃(aq) (2 ml) in a 4 ml amber screw cap vial. The mixture was vortexed (1 min) and allowed to settle
until the phases separated. The aqueous phase was removed and Na₂SO₄ was added to dry the organic layer.
This layer was analysed using GC-FID.

Biodegradation Trials

Additional Odour Compounds vs geosmin-d₃ and 1-CIC₁₀. Murrumbidgee River water (5 l) was collected and
choloroalkanes and labelled geosmin were added to an aliquot (900 ml) as internal standards. This was then
sterilised and analysed as a blank. The remaining river water (4 l) was spiked with the following: 6-
methylhept-5-en-2-one (3560 ng/l); geranyl acetone (3570 ng/l); β-ionone (6280 ng/l) and β-cyclocitril (315
ng/l). The spiked water was then split into two equal portions A and B (2x2 l Schott bottles). To portion
B geosmin-d₃ was added (1000 ng/l) and both bottles were stored at room temperature (22°C) and under
normal light conditions. Sub-samples were taken at ~12 hour time intervals over the next 130 hours and
stripped in duplicate.

Prior to stripping sub-samples from bottle A, a choloroalkane (90 ng/l, 1-CIC₁₀) and geosmin-d₃ (100 ng/l)
were added, whereas prior to stripping sub-samples from bottle B only the choloroalkane (90 ng/l, 1-CIC₁₀)
was added. Response factors for each of the four odour compounds and geosmin-d₃ relative to 1-CIC₁₀
as well as each of the four odour compounds and 1-CIC₁₀ relative to geosmin-d₃ were determined from the
duplicate strips of sub-samples from each bottle at time = 0. These response factors were used to follow the
biodegradation of the four odour compounds added using either 1-CIC₁₀ or geosmin-d₃ as internal standards.

Natural (-)-geosmin vs synthetic (+)-geosmin-d₃. Natural (-)-geosmin produced in culture by Anabaena
circinalis (strain designation 852E) was used to spike Murrumbidgee River water. An aliquot of the A.
circinalis culture (200 ml) was probe sonicated (6x30 sec.) and centrifuged (6000 rpm) for 15 minutes to
clarify. The clarified supernatant was then used to spike raw water.

River water (5 l) was collected and chloroalkanes and labelled geosmin were added to an aliquot (900 ml)
as internal standards. This was then stripped and analysed as a blank. To a portion of the remaining river
water (2 l) all of the clarified supernatant of A. circinalis was added. This solution was mixed and made up
to volume (4 l) with raw water to give a final concentration for (-)-geosmin of 1290 ng/l.

The spiked raw water (4 l) was split into two portions A and B (2x2 l Schott bottles) and portion B was
spiked with geosmin-d₃ to give a final concentration of 1000 ng/l. Both 2 l portions were stored at room
temperature (22°C) and under normal light conditions. A sub-sample (50 ml) was taken from each bottle
preserved with HgCl₂ (40 mg/l) and stored under the same conditions next to the 2x2 l portions. Further sub-
samples were taken at ~ 12 hour time intervals over the next 90 hours, preserved with HgCl₂ and stored in
a cold room (4°C) until ready for stripping. Each sub-sample was stripped in duplicate and each strip
analysed in duplicate by GC-MSD.

Prior to stripping sub-samples from bottle A, a choloroalkane (90 ng/l, 1-CIC₁₀) and geosmin-d₃ (100 ng/l)
were added as internal standards, whereas only the choloroalkane (90 ng/l, 1-CIC₁₀) was added prior to
stripping sub-samples from bottle B. Response factors for 1-CIC₁₀ relative to geosmin-d₃ were calculated at
each time interval from the duplicate strips of sub-samples of bottle A. These response factors were used to determine the concentration of natural (-)-geosmin and synthetic (±)-geosmin-d3 of the sub-samples from bottle B.

RESULTS AND DISCUSSION

Storage of MIB and geosmin in organic solvents

The chemical stability of MIB and geosmin in six commonly used laboratory solvents was evaluated using n-C13 and n-C18 as internal standards (see materials and methods section). The area of each compound was normalised to the area of n-C13. A decrease in this ratio to that obtained for each compound at day 0 indicated a breakdown of the compound. If the internal standard degrades then the ratio of all compounds relative to n-C13 would increase.

All compounds were found to be stable when stored at -15°C in the dark and all except MIB were stable when stored at room temperature (22°C) under normal light conditions. In dichloromethane, MIB degrades (see Fig.1).

![Figure 1](https://iwaponline.com/wst/article-pdf/25/2/115/102702/115.pdf)

**Figure 1** Stability of geosmin, MIB and chloroalkanes in CH2Cl2 at room temperature.

Chemical degradation of MIB

The breakdown of MIB is assumed to be due to acid catalysed dehydration in the presence of HCl formed by the photolytic breakdown of CH2Cl2. A similar breakdown of MIB could be observed by shaking MIB in CH2Cl2 with 1N HCl (see materials and methods section). The disappearance of MIB and the appearance of two earlier eluting breakdown products was also identical with those observed for the original solution (see Fig.2).

The mass spectra of the two breakdown products showed a molecular ion at m/z 150 (168 for MIB) which is consistent with the loss of water and were identical with published mass spectra of 2-methylenebornane and 2-methyl-2-bornene (Martin et al., 1988). Prior to the formation of the two breakdown products the chromatographic peak for MIB was observed to split into two almost co-eluting peaks. The right hand side
of the doublet had the characteristic MIB mass spectrum however the left hand side (LHS) differed. Although the molecular ion was still at m/z 168 for the LHS peak it was lower in intensity and the base peak had changed to m/z 107 from the usual m/z 95. The LHS peak may be 2-methylborneol: the MIB could protonate and dehydrate to a carbocation which could then either lose H⁺ to give the mixture of alkenes or re-hydrate and lose H⁺ to form the epimeric alcohol.

![Figure 2](https://iwaponline.com/wst/article-pdf/25/2/115/102702/115.pdf)

**Figure 2** Acid catalysed decomposition of MIB.

### Biodegradation Trials

**Additional odour compounds vs geosmin-d₄ and 1-CIC₁₀** The suitability of the labelled internal standards for the determination of other odorous algal metabolites was also investigated. Murrumbidgee River water spiked with four odour compounds (see materials and method) was analysed with addition of chloroalkanes, MIB-d₄ and geosmin-d₃ prior to stripping. The labelled compounds were as effective as chloroalkanes when added at the time of stripping giving comparable concentrations for each of the odour compounds. However, as for chlorodecane, the biodegradation rates of all compounds (i.e., target compounds and internal standards) differ sufficiently to preclude addition of the labelled standards at the time of sampling. The rapid loss of the four carbonyl compounds in our river water, also observed for lake water in Germany (Jüttner, 1984), suggests that these compounds may be less troublesome pollutants than MIB or geosmin because they biodegrade more rapidly (see Fig.3).

**Natural geosmin vs synthetic geosmin-d₄** To be suitable as an internal standard that can be added in the field at the time of sampling, the rates of biological loss of a target compound and the internal standard must be identical. In our previous paper (Korth *et al.*, 1991) we showed synthetic MIB-d₄ could be added as an internal standard at the time of sampling. Because the synthetic compound is prepared from a chiral precursor ((+)-camphor), it is optically pure and has the same configuration (-) as MIB from natural sources (Wood and Snoeyink, 1977). Both materials were therefore biodegraded at the same rate. The synthetic geosmin-d₃ was racemic and, when added to a water sample spiked with synthetic geosmin, both materials were biodegraded at the same rate. However, one cannot assume that the rates of biodegradation of (+) and (-) geosmin are the same, and so an experimental study was made of the comparative rates of biodegradation of (-)-geosmin and (±)-geosmin-d₃. Geosmin from natural sources is (-) (Gerber, 1965). An aqueous solution of (-)-geosmin from a culture of *A. circinalis* was used to spike a Murrumbidgee River water sample, together
with (±)-geosmin-d₃, 1-chlorodecane was added as internal standard at the time of analysis. The ratio of m/z 185/182 (molecular ion geosmin-d₃, molecular ion geosmin) increased slowly with storage time (by ca. 10% after 40 h). By measuring the residual concentration of (-)-geosmin and (±)-geosmin-d₃, the residual concentration of (+)-geosmin-d₃ and of (-)-geosmin-d₃ at each storage time can be calculated. Fig. 4 shows that the (+) enantiomer is biodegraded at a slightly lower rate than the (-) enantiomer. Thus, the initial concentrations were: (-)-geosmin 1238 ng/l and (±)-geosmin-d₃ 1049 ng/l (i.e. 525 ng/l of (+)-geosmin-d₃ and 525 ng/l of (-)-geosmin-d₃). After 43 h the concentration of (-)-geosmin had fallen 41%, so the concentration of (-)-geosmin-d₃ must also have fallen 41%. The concentration of (-)-geosmin-d₃ after 43 h = 525 x 0.59 = 309 ng/l. The concentration of (±)-geosmin-d₃ at 43 h = 683 ng/l. Hence the concentration of (+)-geosmin-d₃ at 43 h = 683 - 309 = 374 ng/l. This had the effect of increasing the ratio of m/z 185/182 (and hence lowering the apparent initial concentration of (-)-geosmin in the water) with increasing storage times. However, such errors do not become serious until the geosmin concentration has been greatly reduced by biodegradation during storage and hence does not preclude the addition of the racemic labelled standard at the time of sampling.

Even if the concentration of natural geosmin was allowed to degrade to 5% of the initial value an apparent concentration of 80% of the initial value could still be obtained. If however the degradation was kept below 10%, by storing the sample in the cold and in the dark, an apparent concentration greater than 95% of the initial value is achievable (see Fig.4). This source of error could potentially be avoided by using pure (-)-geosmin as the internal standard (obtained by stereoselective synthesis or by formation and separation of diastereoisomers at a suitable point in the geosmin-d₃ synthesis). Alternatively, a chiral GC column could be used to resolve the (+) and (-) enantiomers of geosmin-d₃ (or even of unlabelled (±)-geosmin).

We planned to test the chiral GC approach by using the labelled racemic geosmin as an internal standard added at the time of sampling to a water sample containing a known concentration of (-)-geosmin. A chiral column (J&W Cyclolex-B) was to be used to separate the optical isomers remaining after storing the water sample for varying times. The rate of biodegradation of the natural (-)-geosmin is the same as that of the (-)-geosmin-d₃ enantiomer of the racemic geosmin-d₃; the more slowly degraded (+)-geosmin-d₃ was expected to give a separate peak and thus not affect the m/z 185/182 ratio of the (-) enantiomer peak. Unfortunately
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initial experiments gave only partial separation of the racemate so the biodegradation trials could not proceed. Despite this, we were able to establish that commercial geosmin (from WAKO) and our synthetic geosmin (both labelled and unlabelled) are racemic. We were also able to establish that natural geosmin as produced in culture by A. circinalis (852E) is optically pure and elutes as the second peak on this column. Because the primary mode of separation of the cyclodextrin columns is by shape recognition (Nowotny et al., 1989) the greater the difference in the shape of one enantiomer to the other the better the separation achievable. The lack of baseline resolution of racemic geosmin on this column indicates that the difference in shape between the 2 geosmin enantiomers is minimal. The reason for this is most likely due to the almost spherical shape of the molecule and the fact that the racemic carbon is towards the centre of the molecule. We are continuing our work in this area in an effort to increase the resolution of the two enantiomers. However even if the resolution of geosmin itself cannot be improved, these chiral columns potentially offer a unique opportunity to quantify other optically pure natural compounds using the opposite enantiomer of the synthetic racemic compound as an internal standard. If the synthetic racemate is labelled, then the same enantiomer could be used to quantify the natural unknown employing mass spectrometry to distinguish between the labelled and unlabelled enantiomers.

![Figure 4](https://iwaponline.com/wst/article-pdf/25/2/115/102702/115.pdf)

**Figure 4** Geosmin biodegradation in Murrumbidgee River water stored at room temperature under normal light conditions.

**CONCLUSION**

We have extensively evaluated the suitability of MIB-d₃ and geosmin-d₃ and believe them to be ideal internal standards for the determination of MIB and geosmin in surface water using CLSA-GC-MS. Both geosmin and MIB may be stored as dilute solutions in acetone, ethanol, methanol, hexane or carbon disulphide for long periods without decomposition. Sunlight decomposes MIB dissolved in dichloromethane to give 2-methylenebornane and 2-methyl-2-bornene.

Standard solutions of geosmin-d₃, or MIB-d₃ in acetone can be added at the time of stripping to determine the concentration of other volatile compounds and provide results which are comparable with those obtained using
chloroalkanes. They can be added at the time of sampling and will compensate for loss of MIB and geosmin during sample transport and storage. Even though some biological discrimination against the synthetic (+) enantiomer of geosmin is evident for our river water, it is not significant unless the sample is allowed to degrade substantially. These labelled compounds would also make excellent internal standards for the determination of MIB and geosmin in other matrices. Their identical physical and chemical behaviour during extraction procedures employed to isolate and concentrate them would mean that the precision and accuracy of the determination is maximized.

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