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# Determination of Geosmin and 2- Methylisoborneol in water using Solid Phase Micro Extraction (SPME) and Gas Chromatography Mass Spectrometry (GC/MS)

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**ABSTRACT**

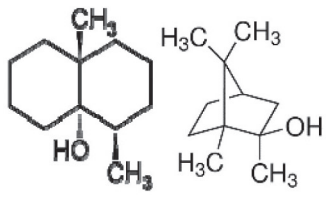
A method for the determination of Geosmin and 2- Methylisoborneol (2- MIB) in water by head space solid-phase micro extraction (SPME) is presented. Polar, Medium Polar and Non-Polar SPME fiber types have been studied for the optimization of Geosmin and 2- MIB extraction from water. Medium polar SPME fiber (DVB/CAR/PDMS) found to be the most efficient SPME fiber type as both compounds are semi volatile odorous compounds. Extraction conditions such as extraction time, sample volume and temperature were also optimized. The best sample size found to be 10 mL, the optimum extraction time is 15 min. and the best extraction temperature is 40 °C. A 3.0 g of Sodium Chloride (NaCl) found to be the best salting out agent. Fiber desorption was carried out at 270 °C for 5 minutes at the GC injection port. Injection port was operated in pulsed splitless mode and Helium was used as the carrier gas at a flow rate of 1.1 mL/min. The selected ions for the quantification of Geosmin and 2- MIB are m/z 112, 125 and m/z 95 accordingly. The results showed good linearity over the concentration ranges of both compounds (R2 values Geosmin - 0.9972, 2-MIB - 0.9980) with a recovery of 91.0% for Geosmin and 92.4% for 2- MIB. Minimum Quantification levels for Geosmin is 3.5 ng/L and that level for 2- MIB is 3.0 ng/L. Minimum Detection level for Geosmin is 1.5 ng/L and that level for 2-MIB is 1.3 ng/L. Both values were below the minimum threshold level for humans where human olfactory system detects the Geosmin and 2- MIB at ~ 5 ng/L.

***Key words:*** *Geosmin, 2- MIB, Solid-phase micro extraction (SPME), Semi volatile, GC/MS*

## Introduction

The provision of safe drinking-water with acceptable aesthetic appearance, related to taste and odour (T and O) is of high priority today (WHO, 2011). Treated pipe born water with detectable unpleasant T and O may be noted by the consumer as unsafe even though it meets all the drinking water standards (Tian, 2013). Drinking water being safe in accordance with health aspects is the primary focus of the water supplier, water consumer generally judges the quality of water by its aesthetic value. Two most common unpleasant T and O causing compounds that affect the aesthetic value of drinking water have been identified as Geosmin (trans-1, 10-dimethyl-trans-9-decalol) and 2- MIB {(1R-exo)-1,2,7,7 tetramethyl bicyclol [2.2.1] heptan-2-ol}. (Manickum and John, 2012). These two compounds are semi volatile tertiary alcoholic compounds with stable chemical structures (Sorial and Srinivasan, 2011).

Geosmin ($C_{12}H_{22}O$) is a bicyclic stereoisomeric organic compound with a molecular weight of 182.31 g/mol and it is a derivative of decalin (decahydronaphthalene/bicyclo [4.4.0] decane). Naturally occurring isomer of Geosmin is (-) with a boiling point of 270 °C (Polak and Provasi, 1992). 2-MIB ($C_{11}H_{20}O$) which is a derivative of borneol, has a molecular weight of 168.28 g/mol. Naturally occurring isomer of 2-MIB is also (-) with a boiling point of 207 ºC (Ju¨ttner and Watson, 2007). Geosmin and 2- MIB provide earthy (muddy) and musty (moldy) taste and odour to water (Ikai *et al.,* 2003; Jiang *et al.,* 2007). Both these odorants originate as secondary metabolites produced from a range of benthic and pelagic microorganisms (Ju¨ttner and Watson, 2007) such as cyanobacteria (Liang *et al.,* 2006; Hikida *et al.,* 2012), actinomycetes (Hikida *et al.,* 2012; 12. Bentley and Meganathan, 1981; Whangchai *et al.,* 2017), fungi (Guttman and Van Rijn, 2008) and amoeba *Vannella* (Hayes *et al.,* 1991) found in source waters such as natural lakes (Izaguirre and Taylor, 1995), reservoirs (Jørgensen *et al.,* 2016) and running waters [6]. However, Tabachek and Yurkowski (1976) [17] reported that cyanobacteria are the main provider of Geosmin and 2-MIB to water than actinomycetes. Furthermore, microorganisms grown in industrial waste treatment facilities, and drinking water treatment plants also produce Geosmin and 2-MIB (Ju¨ttner and Watson, 2007). It has been reported that the human sensory threshold level for Geosmin and 2-MIB are in the range of 5 to 40 ng/L, there- fore, even though there are very low levels present in water, consumers can taste unpleasant earthy and musty odour which directly leading to consumer rejection (AWRF, 2010). Due to the stable chemical structures of both these compounds, conventional water treatment processes such as aeration, coagulation, flocculation, boiling, chlorination and filtration often fail complete removal of Geosmin and 2-MIB (Sorial and Srinivasan, 2011). Hence, occurrence of Geosmin and 2-MIB becomes a challenge to




1. a) Geosmin 1.b) 2. MIB

**Fig. 1.** Chemical structures of 1.a) Geosmin 1.b) 2- MIB

the conventional water treatment processes. Thus, the ability to reliably predict, confirm, and counteract their occurrence would be of immense value, not only to water utilities, but also to other branches of industry, such as aquaculture farms where these compounds can spoil entire harvests and reduce product marketability (Zuo *et al.,* 2009; Jade and Emilia, 2013). Although neither the United States Environmental Protection Agency (USEPA) nor the World Health Organization (WHO) has declared Geosmin and 2-MIB as health hazards, it is reported that cyanotoxins and these two compounds frequently co–occurred indicating odour may work as a warning of the presence of cyanotoxins in water (Chen *et al.,* 2010).

In surface water Geosmin and 2-MIB are existed in two major ways such as cellular (cell-bound) and dissolved fractions and that the differentiation between these two fractions is key to the detection of Geosmin and 2- MIB for effective water treatments. Apart from the treatment-related issues, the dynamics of cell-bound and dissolved fractions also have an crucial domination on the sensory determination of taste and odor (Ju¨ttner and Watson, 2006). Wu and Juttner (1988) clearly demonstrated that particulate Geosmin occurs in cyanobacterial cells as two distinct intracellular fractions, one which is dissolved in the aqueous cytosol and a second which is bound to proteins. The Geosmin fraction which is bound to membrane proteins are integral to the macromolecular protein-pigment photosystem units and the cell degradation by heterotrophic microorganisms liberates Geosmin from the cyanobacterial cell protein matrix (Ju¨ttner and Watson, 2006). Much of the cell bound material can be transferred into the dissolved form by this process because Geosmin and 2- MIB itself is much more slowly degraded by most bacteria than other cell components (Ju¨ttner and Watson, 2006).

Taste and odour issues in drinking water have been a major problem in Sri Lankan water sector for decades. This matter is highly prevalent in some districts in the country such as Anuradhapura, Pollonnaruwa, Ampara, Batticaloa, Trincomalee, Hambanthota, Monaragala, etc…. covering more than five provinces all around the country (Ganegoda *et al.,* 2019). Since Geosmin and 2- MIB compounds are not removable in conventional water treatment processes, the treated water distributed from National Water Supply and Drainage Board, has been rejected by the local consumers due

to the unpleasant T and O. Therefore, an appropriate highly sensitive method is in high demand to analyze Geosmin and 2-MIB from both raw and treated waters. Sri Lanka as a developing country, the major focus was given to find a method which would be relatively inexpensive, sensitive and high sample throughput. Solid-phase microextraction (SPME), a solvent free extraction technique which has gained a lot of significance for the detection of trace compounds in environ- mental samples, appeared to offer these advantages.

So far, there are several methods have been employed to detect Geosmin and 2- MIB in water apart from SPME (Schellin and Popp, 2005; Suffet *et al.*, 2006; Manickum and John, 2012; Ju¨ttner and Watson, 2006). The appropriateness of many methods was compared, considered and studied based on the requirement and the internal mechanisms how Geosmin and 2- MIB release out into the water. Due to the behavioral differences of bound and dissolved intracellular fractions, when designing protocols for extraction and analysis it's very important to consider the influence from above two components (Wu and Jüttner, 1988), Stripping analysis using freeze-thaw (Ju¨ttner and Watson, 2006), sonication (Kilgore, 2006; Thomazeau, 2009), grinding (Mara and Horan, 2009) or nearly saturated NaCl (20%, wt/vol) (Xie *et al.*, 2007) to disrupt cells (e.g., as with the increasingly popular headspace solid-phase micro extraction technique) primarily determines cytosol-solubilized Geosmin (Ju¨ttner and Watson, 2006). Other reported extraction techniques from water matrices include Solvent Extraction (SE) (Schellin and Popp, 2005) and closed loop stripping analysis (Suffet *et al.*, 2006). However, these techniques are all time-consuming and labor-intensive (Manickum and John, 2012). Solid Phase Extraction (SPE) is also time consuming and is unsuitable for low boiling point odorants (Manickum and John, 2012). Since Geosmin and 2-MIB are volatile compounds, Purge and Trap (P and T) method was most frequently used technique. P and T often requires coupling with GC/MS systems operated in Selected Ion Monitoring (SIM). The combination of P and T and GC/MS is effective enough to achieve lower detection limits but the use of P and T in- volves the problem relating to sodium chloride was recorded (Ruana *et al*., 2013). Another popular method is Headspace Sampling (HS), coupled to GC systems. This method allows to introduce volatile compounds directly to GC systems for the analysis of Geosmin and 2-MIB. Solid phase micro extraction (SPME) is another sampling method for direct introduction of volatile compounds into chromatographic systems. SPME is simple and sensitive solvent free extraction technique with the capability of detecting Geosmin and 2-MIB in ppt levels (Fu *et al.*, 2015).

The aim of this study is to develop SPME based method for the detection of Geosmin and 2-MIB below the human threshold level. We report here the development of an optimized SPME method combined with GCMS for the analysis of Geosmin and 2-MIB in water. Our analysis of Geosmin and 2-MIB using SPME is agrees with previous other researchers regarding the SPME analysis as a relatively simple, fast, inexpensive, portable and solvent-free method (Pawliszyn *et al.*, 2012; Vas and Karoly, 2004; Fritsche *et al.*, 2016). Various other reports show excellent analytical utility and applicability of SPME to other common taste-odorants (Ikai *et al.*, 2003; Abdulra'uf *et al.*, 2012).

Prior to the current study, there was no available method in Sri Lanka to detect Geosmin and 2-MIB from water and samples were sent to international laboratories for the analysis. With the completion of the present study, an optimized method to detect Geosmin and 2- MIB from water was constructed using the available instruments/resources in the country while modifying the available literature in the world. This is the first time optimizing a method to detect Geosmin and 2- MIB from water in Sri Lanka and after this study, consultation and sample analysis facilities were provided to National Water Supply and Drainage Board, Sri Lanka to detect Geosmin and 2- MIB from water. Therefore, the current study was an initiation to provide aesthetically safe water to Sri Lankan water consumers aiming high quality water for general public. Thus, the present study aims at optimizing a method to detect Geosmin and 2- MIB using Head space SPME coupled with GC/MS system.

## Materials and Methods

### Chemicals and consumables

Geosmin, 2-MIB and molecular grade Sodium Chloride were purchased from Sigma Aldrich, USA and used as received. Manual fiber assembly of solid-phase micro extraction (SPME) Divinylbenzene/ Carboxen/ Polydimethylsiloxane (DVB/CAR/

PDMS), PDMS and Polyacrilate fibers with various thicknesses were purchased from Supelco (Tokyo, Japan).

**Preparation of samples to employ with Solid-phase micro extraction (SPME)**

**Standards and Calibration Curves**

Geosmin and 2- MIB standards (100 mg/mL in methanol) were dissolved in methanol to make a stock solution at a concentration of 200 $\mu gL^{-1}$. The solution was stored at 4 °C and samples were then prepared in deionized water from the stock solutions in a 100 mL volumetric flask at concentrations of 5, 10, 30, 50, 100 ng/L for Geosmin and for 2- MIB to prepare calibration curves. A 10 mL aliquot of each calibration standards was transferred into 40 mL headspace SPME vial along with 3.0 g of sodium chloride (Sigma Aldrich, USA) and vial was sealed with a twist cap fitted with a Teflon-lined septum.

**Sampling**

Volume of 10 mL surface water from raw water bodies and 10 mL treated water from water treat- ment plants were collected directly into 40 mL headspace SPME vial and vial was sealed with a twist cap. Samples were placed in an ice box (4 °C) and transported to the laboratory. Samples were stored in the dark at 4 °C until analysis performed within 7 days. The water sample was saturated with analytical grade solid Sodium Chloride prior to analysis.

**Extraction of compound in the water using Headspace Solid-phase micro extraction (HS-SPME)**

A 10 mL volume of sample was transferred to a 40 mL headspace vial along with 3.0 g of molecular grade sodium chloride and vial was sealed with a twist cap prior to placement on the vortex machine (1000 rpm) for 1 minute for agitation purpose. Samples were maintained at room temperature until analyzed. The SPME needle pierced the septum of sample vial and the fiber was exposed in the headspace above the sample. The extraction was carried out at different temperatures and different time periods for optimization of the extraction.

**Quantification of Geosmin and 2-MIB using Gas Chromatography–Mass Spectrometry (GC-MS)**

GC–MS analysis was carried out with Agilent Model 7890A Gas Chromatograph and Agilent Model 5890 C Mass Spectrometer Cross-linked HP-5MS (30 m × 250 µm × 0.25 µm film thickness) fused-silica capillary column with 5% Phenyl Methyl Siloxane was used. The GC operating conditions were as follows: Inlet helium carrier gas flow rate was maintained at 1.1 ml/min. Injection port was operated in pulsed splitless mode and was fitted with 0.7 mm id SPME injection liner (Sigma Aldrich, USA). Head pressure was set to 9.35 Pa of Helium for 1.30 minutes, then changed to a constant flow of 1.1 ml/min to give a velocity of 38.41 cm/s. Oven was initially held at 60 °C for 1 minute, then increased by 10 °C/min to 300 °C and held for 4 minutes. The mass spectrometer conditions were as follows: MS source temperature, 230 °C, MS Quadropole temperature, 150 °C; ionizing voltage, 70 eV. The full scan mass spectra were obtained at an m/z range of 33–550 D. Quantification was performed by external calibration method by integrating the correlation area of the peak.

**Application of the optimized method for the detection of Geosmin and 2-MIB in raw and treated water samples**

The optimized method was applied to several raw and treated water samples collected from source water and water treatment plants. Geosmin and 2-MIB in the water samples were identified by comparing mass spectra with NIST reference mass spectral library.

## Results and Discussion

**Optimization of headspace solid-phase micro extraction (HS-SPME)**

When optimizing extraction conditions in any SPME method there are a number of variables which must be considered. The major factors to be considered include: extraction mode (i.e., direct vs. headspace sampling); salt concentration; sample volume; fibre coating, extraction time and temperature (McCallum *et al.,* 1998). Each of these parameters were examined systematically to find the optimum conditions for extraction. All extractions were performed for 10 min, at ambient temperature for immersion sampling and at 30 °C for headspace sampling initially.

**Sample Volume**

Initially a sample volume of 1 mL (triplicate

samples were used) was selected at a level of 100 ng/L (ppt) referring to the available literature (Ju¨ttner and Watson, 2007), but it was not detected at GC/MS scan mode. Then concentration was increased to 500 ng/L (ppt) (triplicate samples were used), But could not detect any peak at scan mode. Finally, concentration was increased to 200 µg/L (ppb) and Geosmin and 2- MIB peaks were detected at scan mode (triplicate samples were used). For 200 µg/L at 1 mL standard sample, abundance of the peaks was very low at sim mode. How- ever, no peak was detected for ng/L (ppt) levels at scan mode with 1 mL sample size. Further at both sim and scan modes, at 1 mL sample size, none of the levels of calibration series (From 5 ng/L to 100 ng/L) of the calibration curve were detected (triplicate samples were used). Therefore, sample volume was changed to 10 mL and standard peaks of Geosmin and 2- MIB were obtained at scan mode at 500 ng/L level. The peak abundance also was significantly higher than the 1 mL sample size ($p< 0.05$). Therefore 10 mL ± 2.43 sample size was selected as the best sample size. A larger sample volume more than 10 ml was not tested in order to minimize the wastage of chemicals since both Geosmin and 2- MIB stan- dards are very expensive. The efficiency of headspace extraction was examined as a function of the sample volume at a fixed headspace: sample volume ratio (4:1). Interestingly there was a decrease in the percentage extracted with increasing sample volume. The decrease in extraction efficiency on increasing the sample volume might be offset by the increased mass of material available for extraction in the larger samples. Using a smaller headspace: sample volume ratio (1: 4), more efficient extractions could be obtained at higher sample volumes, thus maximizing the mass of material on the fibre. This agrees with findings of McCallum *et al.* (1998) (McCallum *et al.*, 1998).

**Extraction temperature**

The incubation temperature of sample is one of the most influential parameters on the sensitivity for the HS – SPME sampling, but it cannot be set beyond the boiling point of sample medium (Ikai *et al.*, 2003). In this study, six different temperatures were tested based on the literature (Chang *et al.*, 2008) as 28°C, 30°C, 40°C, 50°C, 60°C and 70°C (Figure 2). Highest yield of Geosmin and 2- MIB levels were obtained at 40 °C± 8.34 temperature. (Figure 2). When increasing extraction temperature higher than 40 °C, higher amount of water vapor comes up more to the gaseous stage. Geosmin and 2-MIB are soluble in water at this level and they produce very low vapor pressures (Ikai *et al.*, 2003). Therefore, water vapor competes with Geosmin and 2- MIB and binds to the SPME fiber more and more. As a result, amount of Geosmin/2- MIB molecules bind to the fiber becomes lesser. Therefore, extraction temperature at 40 °C yielded highest extraction efficiency. Moreover, heating is a technique used to improve releasing of intracellular fractions of Geosmin and 2- MIB (Ju¨ttner and Watson, 2007). Hence extraction temperature 40 °C yielded better than 30 °C since heating is up to some point is required. Therefore, optimized extraction temperature was set as 40 °C.

The variation of adsorption with temperature is a consequence of the sensitivity of both the water–headspace and headspace–fibre partition coeffi-

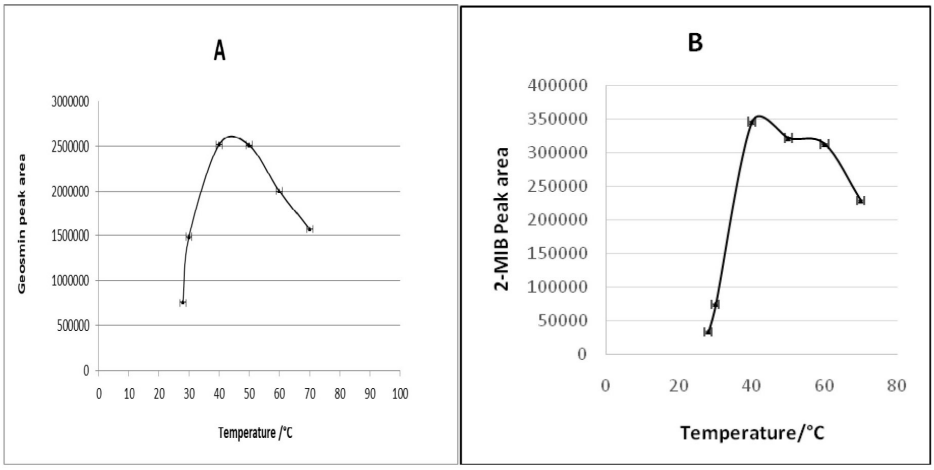



**Fig. 2.**Adsorption temperature profiles for A) Geosmin B) 2-MIB

cients to temperature (McCallum *et al.*, 1998).

**Vortexing Rate**

Vortexing rate is required to break the cyanobacterial cells and releasing of cell bound (compounds in the cytosol) Geosmin and 2- MIB into the water. Furthermore, it's important to dissolve Sodium Chloride evenly in the sample. All samples were stirred so as to produce the agitation necessary for efficient transfer of the analyte from the aqueous phase to head phase (McCallum *et al.*, 1998). Agitation rate became constant by stirring at more than 1000 rpm, but faster agitation tends to be uncontrollable and might cause a poor measurement precision. Therefore, the vortexing rate settled as at 1000 rpm (Saito *et al.*, 2008).

**Salting out agent**

The sensitivity of the HS- SPME analysis further depends on the addition of salts to the sample that is used for salting out the analyte by reducing the solubility. In an attempt to vary the addition amounts of Sodium Chloride to the sample, the maximum effect was observed when the sample was saturated with it (Ju¨ttner and Watson, 2007). Use of 3.0 g of sodium chloride (NaCl) that is necessary amounts to saturate 10 mL of water found to be the best salting out agent which is also agrees with Hurlburt *et al.*, 2009 (Hurlburt *et al.*, 2009). Further NaCl was important to cyanobacterial cell lysis and releasing of cell bound (compounds in the cytosol) Geosmin and 2- MIB into the water. More importantly, without addition of sodium chloride, Geosmin or 2- MIB was not detected (Triplicate samples were tested). This fact is agreed with previous studies where Saito *et al.*, (2008) recorded that extraction yield also increased in 12 times by a salting out effect.

**SPME Extraction time**

Having established the best extraction conditions, the influence of fibre coating and the effects of extraction time were examined using the conditions derived earlier (10 mL sample of in 40 mL vial saturated with 3.0 g NaCl, 1000 rpm stirring, headspace sampling).

Eight different extraction times were used based on the literature (Chang *et al.*, 2008) and Geosmin and 2- MIB peaks were obtained accordingly (Figure 03).

According to Figure 03 A, the first highest Geosmin and 2- MIB peak area have been obtained at extraction time of 15 minutes ± 10.65. 5-minute extraction might be not enough for the compounds to completely adsorb into the SPME fiber. Beyond 15 minutes when increasing the time duration, peak area increased. But at 40-minute point, there were no significant difference between 15-minute extraction time and 40-minute extraction time correlation area ($p > 0.05$) for both the compounds. However, when time increased beyond 40 minutes, until 100 minutes, the correlation area slightly increased. But a shorter time duration was preferred for the analysis, hence 15 minutes was selected as the SPME extraction time.

**SPME fiber type**

Various SPME fiber types (Polar, Non polar and Medium polar) have been tested for Geosmin and 2-MIB extraction efficiency from water. Medium polar SPME fiber (divinylbenzene/ carboxen / polydimethylsiloxane (DVB/CAR/PDMS)) microfiber with film thickness 50 µm) proved to be the most efficient for Geosmin and 2- MIB extraction

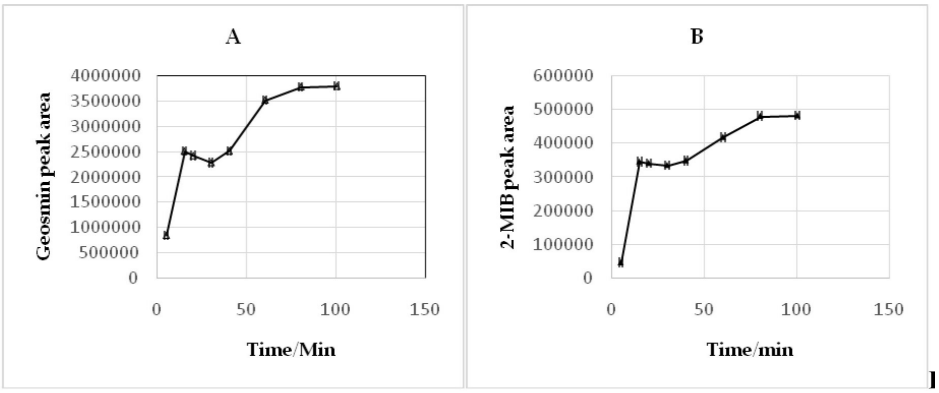



**Fig. 3.** Adsorption time profiles for A) Geosmin B) 2-MIB

as they are semi volatile odorous compounds. Both Geosmin and 2- MIB are rather non-polar compounds with molecular weights of 182.307 g/mol and 168.28 g/mol accordingly. However, no standard peak was obtained at GC/MS Scan mode for both compounds when testing with Polyacrylate (Polar) and PDMS (Non-polar) SPME fibers. (Triplicate samples were tested). Geosmin and 2- MIB standard peaks were only obtained at GC/MS Scan mode with DVB/CAR/PDMS (Medium Polar) fiber type. To select the most suitable medium polar SPME Fiber type for the present study, two different Medium polar fiber types; a (divinylbenzene/ carboxen (DVB/CAR)) and a (divinylbenzene/ carboxen/polydimethylsiloxane (DVB/CAR/ PDMS)), were compared in their ability for adsorbing Geosmin and 2-MIB. Both fibers were almost the same in the performance of retention, suggesting that they can be used effectively for Geosmin and 2-MIB sampling. Both of these fibers produce standard peaks at GC/MS Scan mode, however standard peaks obtained by (divinylbenzene/ carboxen / polydimethylsiloxane (DVB/CAR/PDMS)) was obtained at a lower retention time and correlation area was higher when compared to (divinylbenzene/ carboxen (DVB/ CAR)) fiber type for both Gosmin and 2- MIB compounds. Therefore DVB/CAR/PDMS fiber type found to be the most effective for the analysis of Geosmin and 2- MIB. Triplicate samples were tested for all the steps.

The optimized extraction conditions used in subsequent work are summarized as:

**Optimization of GC/MS conditions**

The GC analysis of Geosmin and 2-MIB was performed on a fused-silica capillary column (HP-5MS) under the condition of programmed temperature and quadrupole MS was used for the detection. After SPME fiber desorbed at 270 °C, 5 min, fiber was then baked for an additional 4 min in an external heating block to prevent carryover. Noticeably, Geosmin and 2- MIB extracted on the fiber was completely desorbed within 5 min by heating in the GC-injection port at 270 °C and carry over was not observed because the fiber was washed during exposition. The Geosmin and 2- MIB peaks were not detected by re-exposition of the fiber after heating and this agrees with Saito *et al.,* 2008 (Saito *et al*., 2008). Therefore, fiber could be used for the next sample reassuring that there is no any compound from the previous sample retained in the fiber. Fiber was conditioned each day according to the manufacturer's specifications prior to sample analysis in order to

**Table 1.** Optimized sample preparation for Geosmin and 2-MIB

| Purpose | Optimized Condition |
|---|---|
| mixing | vigorous agitation |
| extraction mode | headspace sampling (solution saturated with NaCl) |
| sample volume | 10 ml sample in 40 ml vial |
| fibre coating | DVB/CAR/PDMS |
| extraction time and temperature | 15 min at 40 °C |

**Table 2.** Supplied and optimized conditions at GC/MS

| Parameter | Supplied Condition |
|---|---|
| Injection port | pulsed splitless mode |
| Injection liner | 0.7 mm id |
| Head pressure | 25 Pa of Helium - 1st minutethen constant flow of 1.1 ml/min |
| Velocity | 40 cm/s |
| Capillary Column | 30m, DB -5MS |
| Stationary Phase | 0.25mm i.d.and a 0.25 µm film thickness |
| Carrier Gas | Helium |
| Carrier gas flow rate | 1.1 ml/min. |
| Oven temperature programme | 60 °C – 1st minutethen increased by 10 °C/min to 300 °Cheld for 4 minutes. |
| Electron ionization energy | 70 ev |
| Selected ion for quantification | 2- MIB - m/z 95 and m/z 125GSM - m/z 112 |
| Alternative monitoring dwell times | 1001.ts |

prevent carryover from previous day samples. Both Full- Scan and Sim modes were used in this method. Under these optimized conditions, the compounds were successfully separated within 11 min without any interfering peaks on the chromatogram. Moreover, it was noticed that it's essential to operate injection port in pulsed splitless mode using a splitless liner for SPME analysis. Further selected ions of the base peaks and molecular ion for Geosmin were m/z 112 and 125 and for 2- MIB were m/z 95 and they were monitored alternatively at dwell times of 1001.ts each. The total required time for the sample analysis including HS-SPME sampling was about 26 min. The amount of Geosmin and 2-MIB extracted by HS/SPME method was calculated by comparing correlation area of the peak. All the important supplied conditions and the optimized sampling conditions at GC/MS has been given in the Table 2.

**Calibration Curves**

Excellent linearity was obtained for both Geosmin and 2-MIB, with R2 values of 0.9972 and 0.9980, respectively, over a range of concentrations from 5 to 500 ng/L (Figures 6). The correlation area was measured to construct calibration curve and to determine concentration of Geosmin and 2- MIB in samples. Seven replicates were analyzed for method

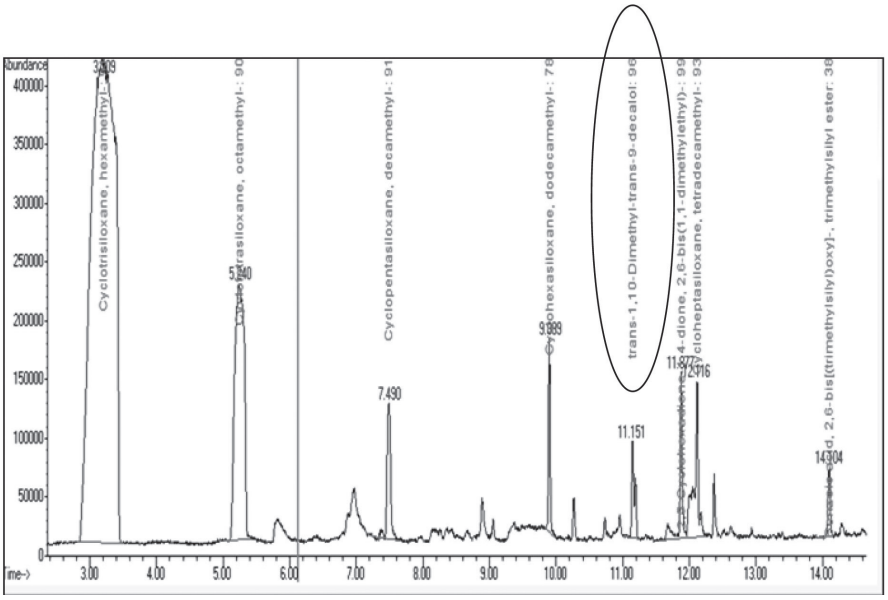



**Fig. 4 a.** Total ionic current spectrum of Geosmin standard (Retention Time 11.15 min)

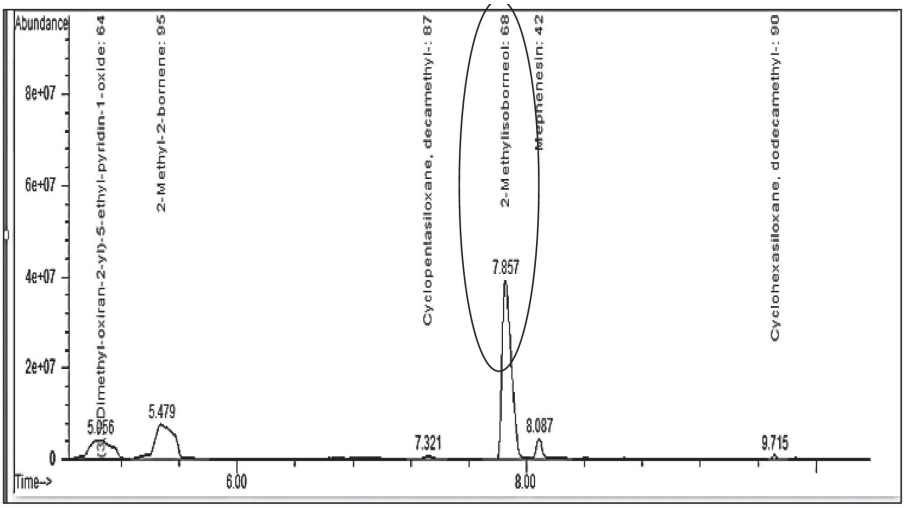



**Fig. 4b.** Total ionic current spectrum of 2- MIB (2-Methylisoborneol) standard (Retention time is at 7.85 min)

detection limits (MDL) and for Geosmin MDL was calculated as 1.5 ng/L whereas for 2- MIB, MDL was calculated as 1.3 ng/L. For Geosmin, Minimum Quantification level (MQL) was calculated as 3.5 ng/L whereas for 2- MIB, MQL was calculated as 3.0 ng/L. Both of the MDL and MQL values are less than human sensory threshold levels of both Geosmin and 2- MIB. Excellent sensitivity and chromatographic performance were demonstrated across the calibration range for both Geosmin and 2-MIB.

**Recovery test**

When analyzing Geosmin and 2- MIB levels in water, there is a possibility that the analytes might be adsorbed onto the walls of the vial, lost into the small headspace just beneath the cap and onto the Teflon-lined septum itself. A volume of 100 mL deionized water spiked with Geosmin and 2-MIB at the level of 100 µg/L(ppb) and replicate aliquots of 10 mL were subjected to HS- SPME analysis in SIM and Scan mode. Interfering peaks were scarcely observed on the resulting SIM and Mass Chromatograms of each compound. The recovery was obtained as 91.0 % for Geosmin and 92.4 % for 2- MIB and the Mass Spectra were agreed well between this recovery tests. Final results indicated that the satisfactory recoveries along with excellent detection limits. However, the desired levels of Geosmin and 2- MIB were not able to measure using Scan mode since minimum human threshold levels of these two odorants are very low levels low as ng/L levels. Therefore, using the obtained retention times, Sim mode was employed for the determination of Geosmin and 2- MIB levels. Under the optimized conditions, retention time of 2-MIB and Geosmin were 7.85 min and 11.15 min respectively.

**Detection and quantification of Geosmin and 2-MIB**

A 500 ng/L Geosmin and 2- MIB extracted by HS/SPME method was used for identification of

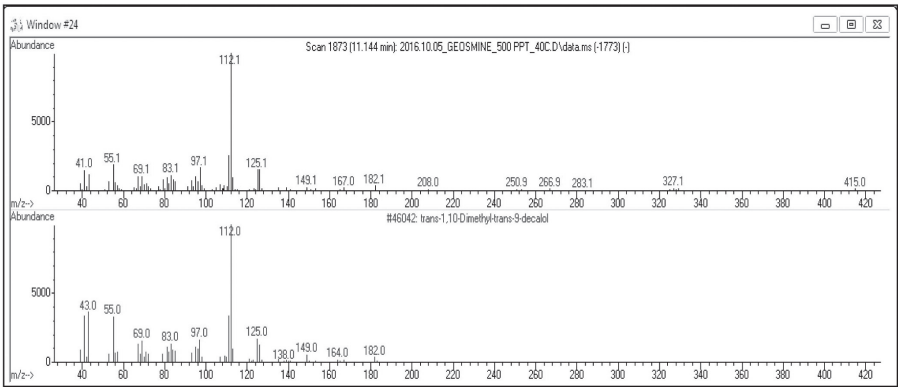



**Fig. 5a.** Comparison of mass spectra of Geosmin peak at 112 m/z with NIST library spectra

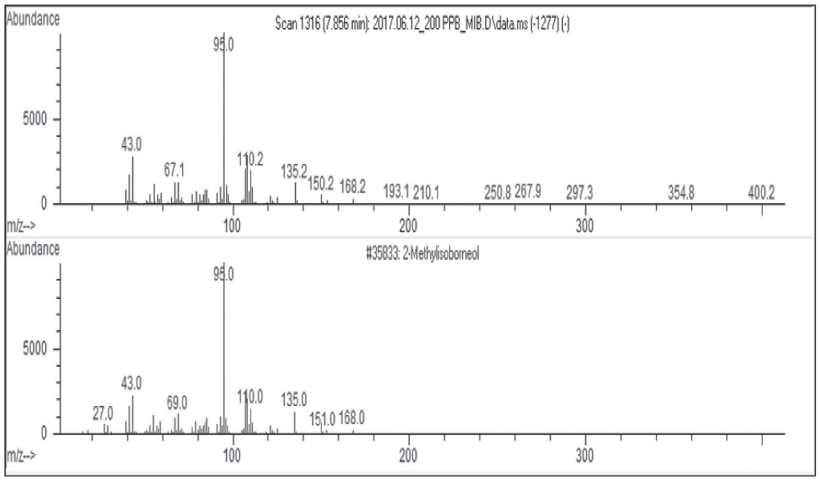



**Fig. 5b.** Comparison of mass spectra of 2- MIB peak at 95 m/z with NIST library spectra

Geosmin and 2-MIB via Scan mode at GC/MS as per figure 4a and 4b, which are demonstrating the total ionic current spectrum (TIC spectrum). At a retention time of 11.15 minute and 7.857 min Geosmin and 2- MIB peaks were appeared accordingly. Mass spectra of Geosmin peak and 2- MIB peak (actual spectrum) were matched with NIST library spectra (reference spectrum) (Figure 5a & 5b). Since for the both compounds, the human sensory threshold level is very low as ng/L level (5 ng L-1), Sim mode was used for the further analysis. The selected ions for the quantification of Geosmin are of m/z 112 and 125 and for 2- MIB is of m/z 95. Both Geosmin and 2- MIB were eluted as single and symmetrical peaks at 11.15 min and 7.857 min respectively and both the compounds gave excellent response to GC–MS-SIM detection.

**Application of the optimized method for the detection of Geosmin and 2- MIB in raw and treated water samples**

The optimized method was finally applied for the determination of Geosmin and 2-MIB in various water resources across the country. Analyzed Geosmin and 2- MIB levels in raw and treated water resources are given in the Table 03. The Geosmin levels in raw water ranges from 7.8 ng/L ± 3.27 to 34.6 ng/L ± 1.32 and that of treated water ranges from 8.2 ng/L ± 0.22 to 38.9 ng/L ± 1.34. The 2-MIB levels in raw water ranges from 8.2 ng/L ± 0.53 to 139.4 ng/L ± 6.20 and that of treated water ranges from 3.5 ng/L ± 0.42 to 22.7 ng/L ± 3.47. At some raw and treated water samples, Geosmin and 2-MIB were below the minimum detection level. All the sample were collected in the dry season of the area where water levels of many reservoirs remains low.

According to the recorded data in table 03, highest Geosmin level in raw water was detected at Sagama tank (34.6 ng/L ± 1.32) whereas at Kanthale tank Geosmin was not recorded beyond the minimum detection level of Geosmin (1.5 ng/L). Highest Geosmin level in treated water was detected at Sagama Water Treatment Plant (WTP) (38.9 ng/L ± 1.34) whereas from treated waters of Kondawatuwana and Unnichchi WTPs, Geosmin was not detected. Furthermore, highest 2-MIB level in raw water was detected at Kondawatuwana tank (139.4 ng/L ± 6.20) whereas lowest 2-MIB level in raw water was detected at Thuruwila tank (8.2 ng/L ± 0.53). Moreover, highest 2-MIB level in treated waters was detected at Jayanthi WTP (22.7 ng/L ± 3.47) whereas 2-MIB was not recorded in Kondawatuwana and Unnichchi WTPs. When analyzing this result, it's a clear observation that all the treated water samples apart from few exceptions, Geosmin and 2- MIB levels were higher than that of raw water sample. Some water processing steps may cause that elevation level of Geosmin and 2-MIB which again lead to potential consumer rejection of treated water. Geosmin and 2-MIB are secondary metabolites of a range of cyanobacteria and algae and in raw water and it is present both in solution and in a suspended form mostly associated with the host cyanobacteria (Ashitani *et al.*, 1988). According to Ashitani (1988), Geosmin and 2-MIB in suspended form were well removed by coagulation and sedimentation alone. Geosmin and 2-MIB

**Table 3.** Detected Geosmin & 2-MIB level in raw and treated water at Dry season

| Water Sources | Detected Geosmin Level | | Detected 2-MIB Level | |
|---|---|---|---|---|
| | Raw Water (ng/L) | Treated Water (ng/L) | Raw Water (ng/L) | Treated Water (ng/L) |
| Jayanthi tank | 20 ± 3.50 | 18.6 ± 0.25 | 97.3 ± 6.29 | 22.7 ± 3.47 |
| Sagama tank | 34.6 ± 1.32 | 38.9 ± 1.34 | 57.9 ± 3.11 | 4.5 ± 1.80 |
| Kondawatuwana tank | 12.4 ± 3.60 | N.D. | 139.4 ± 6.20 | N.D. |
| Unnichchi tank | 8.3 ± 1.91 | N.D. | 25.8 ± 0.43 | N.D. |
| Kanthale tank | N.D. | 8.4 ± 3.65 | 26.2 ± 7.11 | 3.5 ± 0.42 |
| Kala tank | 8.2 ± 1.55 | 10.3 ± 7.32 | 9.5 ± 0.51 | 11.2 ± 8.45 |
| Nallachchiya tank | 7.8 ± 3.27 | 8.2 ± 0.22 | 11.7 ± 5.22 | 11.9 ± 1.81 |
| Thuruwila tank | 8.1 ± 0.97 | 8.5 ± 3.21 | 8.2 ± 0.53 | 10.3 ± 1.32 |
| Thissa tank | 8.8 ± 1.77 | 11.1 ± 0.42 | 16.4 ± 0.28 | 20.3 ± 3.87 |
| Nuwara tank | 10.9 ± 3.24 | 11.2 ± 4.21 | 20.2 ± 4.14 | 22.5 ± 0.52 |
| Padawiya tank | 10.8 ± 6.23 | N.A. | 46.3 ± 3.58 | N.A. |

N.D. – Not Detected, N.A. – WTP Not Available

present in solution could be removed almost to an undetectable level in the rapid sand filter where no pre-chlorination was practiced. But when raw water enters to specific processing steps at water treatment plant, such as pre-chlorination, cyanobacteria cells disintegrates and cell lysis occurs (Ashitani et al., 1988). At that point Geosmin and 2-MIB leaks out to the water and leads to an elevated level of Geosmin and 2-MIB in treated water than the level in corresponding raw water. AT Kondawatuwana WTP, Unnichchi WTP and Jayanthi WTP, treated water Geosmin and 2- MIB levels were less than respective raw water Geosmin and 2-MIB levels. When water treatment processes are analysed, Unnichchi WTP had Granu- lar Activated Carbon beds (GAC) and Kondawatuwana WTP used Powder Activated Caron (PAC) for its regular water treatment processes. Jayanthi WTP also used PAC time to time when the odour and taste outbreaks occurred at dry season. Therefore, these results might evidence that activated carbon is a potential solution to remove Geosmin and 2-MIB from treated water used at water treatment plants. Moreover, Powder Activated Carbon (PAC) and Granular Activated Carbon (GAC) are widely used around the world to remove Geosmin and 2-MIB in water (Drikas et al., 2009).

When considering headspace sampling, it proved to be more efficient (40% extraction) than immersion sampling (10% extraction) and the extraction efficiency was further improved by saturating the sample with NaCl (60% extraction). This was agreed with McCallum et al., (1998). Moreover, performance data show that the method is suitable for determinations of Geosmin and 2- MIB in drinking water and surface water, at levels below the odor threshold values. Furthermore, SPME is simpler, less labour and time intensive and relatively inexpensive to set up and run for the analysis of Geosmin and 2-MIB. In addition, the method has the potential for automation, which would further increase sample throughput and decrease analysis costs (McCallum et al., 1998). For consumables, whether operating in manual mode or with an auto sampler, 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 is additional). However, SPME method offers only a single order of magnitude in concentration and limited to a single injection per sample. Also, HS-SPME is not suitable for auto- mated analysis because of the problems in the sta- bility and durability of SPME probe (Hurlburt et al., 2009). Considering all the facts it can be inferred that SPME could be the method of choice for applications that require detection of the compounds at the levels below hu- man detection and it is very suitable to use in Sri Lanka. After optimizing this method, for the first time in Sri Lanka, Geosmin and 2- MIB were de- tected from water (both raw and treated) and con- sultations and sample analysis facilities were pro- vided to National Water Supply and Drainage Board, Sri Lanka to increase drinking water quality of Sri Lankan general public.

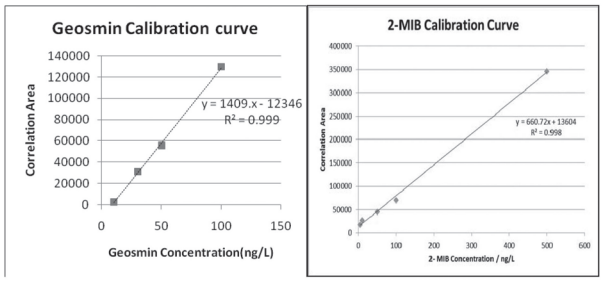



**Fig. 6.** Calibration curve for A: Geosmin, B: 2-MIB for quantification

## Conclusions

Solid-phase micro extraction integrates sampling, extraction; concentration and sample introduction into a single solvent-free step and analytes in the sample are directly extracted and concentrated to the extraction fiber. The current method is largely modified and optimized method and is a simple, sensitive, cost effective and largely reduces the sample preparation time. Further it can be directly applied to the analysis of a little volume of environmental water samples without any pretreatment. This method can be recommended to use for routine analysis of supplied water since it takes a very less sample analysis time. Therefore, this method provides a useful tool for the screening and determination of Geosmin and 2- MIB in water analysis for the first time in Sri Lanka and it is very appropriate to be used in Sri Lanka when considering cost, time and sensitivity.

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