

The Determination of Geosmin and 2-Methylisoborneol Using Stable Isotope Dilution Purge-and-Trap Gas Chromatography Mass Spectrometry

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Abstract: An isotope dilution technique based on purge-and-trap coupled to gas chromatography with mass spectrometric detection were developed and optimized for the trace determination of the typical odorous compounds; Geosmin (GSM) and 2-methylisoborneol (MIB). The selection of quantitative ion and the factors of programmed temperature, the sample purge temperature, salinity on the extraction of odor compounds were discussed. D₅-Geosmin and d₃-MIB as the isotopes internal standards are added to the 25ml of water sample containing sodium chloride (20%(m/v)), and the analytes were purged at 60°C by a flow of N₂ in 13min and trapped on a Tenax-Silica Gel-Charcoal sorbent, then separated and determined by gas chromatograph coupled to a mass spectrometer. Under the optimal conditions, the calibration curves of MIB and GSM were linear in the range of 5–500ng/L, with a correlation coefficient above 0.9998. The limits of detection (LOD) were 4.12ng/L for MIB and 3.60ng/L for GSM. Recovery were 82%~95% for GSM and 99%~101% for MIB, and precision were 5.20% for GSM and 0.78% for MIB at 100ng/L from a reservoir source water sample in Fuzhou, China. This method was applied to the analysis of environmental water samples successfully.

Keywords: Stable isotope dilution; Purge-and-trap; Odorous compounds; Gas chromatography–mass spectrometry

1 Introduction

In recent years, taste and odour problems in drinking occurrence in home and abroad frequently^[1,2], more and more impact on the quality of drinking water and aquatic products^[3], thereby affecting aquatic life and human health^[4,5]. Geosmin (GSM) and 2-methylisoborneol (MIB) have been known to be the main semi-volatile compounds which impart an earthy–musty odour in water at very low odour thresholds; as low as 4ng/L for GSM and 15ng/L for MIB^[6]. The limits of MIB and GSM was only 10ng/L in the “standards for drinking water quality” (GB5749 - 2006) in China^[7]. Thus, with the improvement of living standard and the concerns on their own health, the study on the odorous compounds in drinking water has become a research hotspot. Therefore, it is particularly urgent and important to have techniques available which allows for the trace determination of these compounds for the presentiment of the odour emergencies and the adverse human health effects.

About the analytical methods, in home and abroad, focus on instrumental analysis method, that is, the water samples is pretreated firstly, and then sent to GC/MS for qualitative and quantitative detection^[8]. Purge and Trap is one of the common pre-treatment methods, and is listed as the standard analytical methods in EPA^[9]. Amir

et al. adopted an automated technique based on purge-and-trap (P&T) coupled to gas chromatography with mass spectrometric for the detection of five of the most important ground water odorants^[10], but, isotope dilution technique was not added compensate for the variability in the extraction process in this paper. Isotope dilution technique is one of the most accurate analysis methods which can effectively eliminate the loss of analytes in the handling process, the matrix effects and signal drift and other factors that influence the accuracy of that^[11]. The P&T-GC/MS coupled with isotope dilution for the determination of MIB and GSM in home and abroad was not available. In this paper, an isotope dilution technique based on purge-and-trap coupled to gas chromatography with mass spectrometric detection were developed and optimized for the trace determination of GSM and MIB, and applied to the analysis of environmental water samples and the algal culture successfully.

2 Experimental

2.1 Equipment and Reagents

Standards of GSM and MIB were purchased from Sigma-Aldrich (USA) as solutions of 100mg/L in methanol. Fresh mixed standard stock solutions of 1mg/L

in methanol should be prepared before extraction. Internal Standards of (+/-)-d₅-geosmin(d₅-GSM) and (-)-2-methylisoborneol-d₃(d₃-MIB) were gained also from Sigma-Aldrich. Sodium chloride is baked at 600 °C for 6h before use; Methanol was of the HPLC grade, provided by MERCK; The dilution water is produced from a Millipore Direct-Q3 Water System.

2.2 P&T Method

An OI-Analytical Eclipse (MODEL: 4660) was used. Target compounds together with internal standards are purged from 25ml of water sample containing sodium chloride (20%(m/v)) at 60°C by N₂ (40mL/min) in 13 min and trapped on a Tenax-Silica Gel-Charcoal sorbent. After that, trap temperature is raised to 200°C for 0.5min to desorb the analytes. Following this step, the trap was baked at 240°C for 10 min. This process was enough to decrease cross contamination.

2.3 GC/MS Method

A gas chromatogram quadrupole mass spectrometer (Agilent GC 6890/5973i MSD, USA) is utilized for component separation and detection. A 30m×0.25mm I.D. DB-5MS fused silica capillary column (J&W Scientific, Agilent, USA) with a 0.25µm film thickness was used. The injector was set at 280°C in the split mode (split ratio:20:1), and He flow rate is maintained at 1.0mL/min. The oven temperature is programmed from an initial temperature of 50°C held for 2min, then ramped at 5°C/min to 160°C, finally ramped at 20°C/min to 280°C held for 8 min, and the total run time was of 38min. The transmission line (AUX)、ion source and quadrupole temperature were separately set at 300°C, 230°C and 150°C; electron ionization was performed at 70eV; acquisitions were performed in SCAN mode from 45 to 200 amu for identification purposes and in selected ion (SIM) mode for quantification. The mass spectral quantitative ions were 107、138、112、114 for MIB、d₃-MIB、GSM、d₅-GSM.

3 Results and Discussion

3.1 Qualitative Analysis and the Selection of Quantitative Ions

The ion chromatogram in SIM mode of MIB, d₃-MIB, GSM and d₅-GSM are shown in Figure1.. It shows that, the retention time (RT) interval of standards and internal standards is immovable. Acquisitions were identified according to the retention time and the ion chromatogram in SCAN mode and quantified according to the peak area ratio of standard and internal standard in SIM mode. The ions monitored by SIM were m/z 107, 135, 150 for MIB; 138, 110, 153 for d₃-MIB; 112, 125 for GSM; and 114,

128 for d₅-GSM. The quantitation ions were m/z 107、138、112、114 for MIB、d₃-MIB、GSM、d₅-GSM. From figure 2.[¹²], we can see that, 95 was not selected as the quantitative ion for MIB. The reasons are as follows; firstly, the highest abundance of d₃-MIB and MIB fragment are both 95, if 95 is chose, it will cause a great interference. Secondly, in the 95 fragment route, which undergoes a ring opening and subsequent elimination of acetone giving the base peak at m/z 95. The base peak cannot be used for quantitation because the label is retained in the acetone fragment.

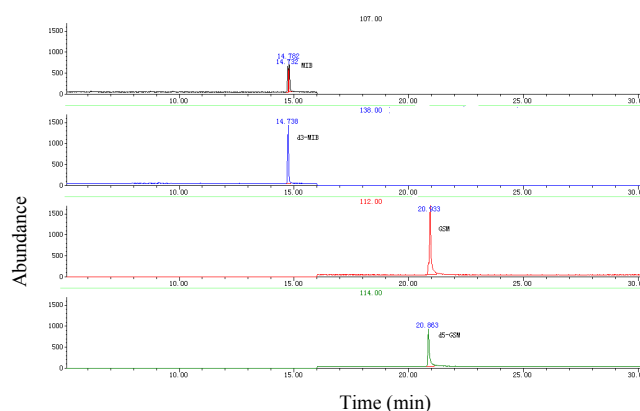


Figure 1. The ion chromatogram in SIM mode of analytes

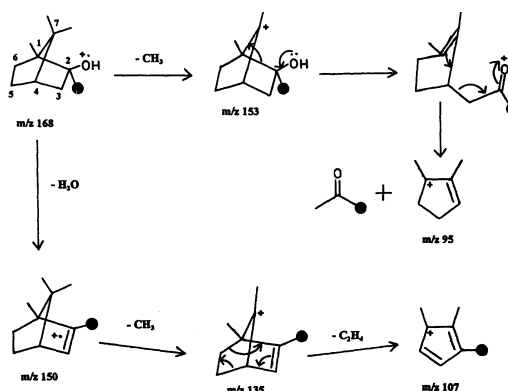


Figure 2. Proposed fragmentation scheme for d₃-MIB

3.2 Optimization of Temperature Program

Gas chromatograph temperature program exerts a great influence on the separation of analyte[¹³]. To improve separation, five temperature program are adopted, and theoretical plate number was compared based on theoretical plate number. The results were shown in Figure 3..

As shown in Figure 3., the theoretical plate number of 5°C is higher than several other temperature program, which indicates that 5°C have a better separation for both of these two compounds[¹³]. At the same time, the retention time interval of two compounds increases from 1.177min to 6.183min, with the heating rate decreases.

For the relatively complex sample matrix, it is more conducive to exclude interference. Therefore, the optimal temperature program is set at 50°C(2min) and then raised at 5°C/min to 160°C, and then ramped at 20°C/min to 280°C where it was maintained for 8min.

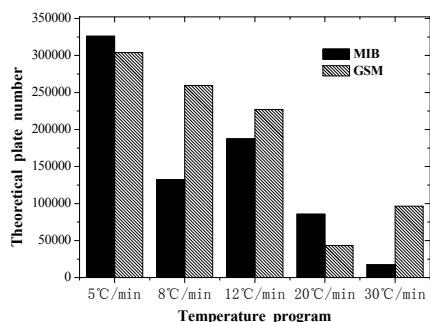


Figure 3. The theoretical plate number of different temperature program

3.3 Sample Purge Temperature

Heated samples can increase the dissociation energy of odorous compounds from the solution, thus accelerating the mass transfer process^[14]. The effect of sample purge temperature on sensitivity is shown in Figure 4.

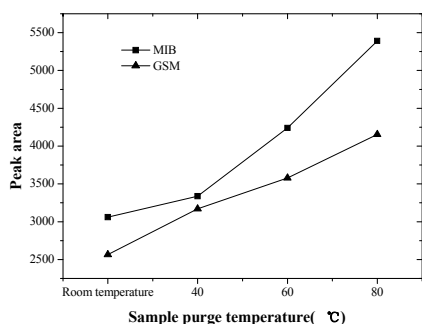


Figure 4. The effect of sample purge temperature

As shown in Figure 4., the peak area of the two compounds increased with the temperature increasing. The sample purge temperature can speed up the molecular motion, resulting in the increase of the liquid vapor pressure, and is conducive to adsorption. But if the temperature is too high, it may lead the water vapor entering into the pyrolysis pipe, affecting the gasification of sample, then affecting the following-up adsorption and determination of target. Meanwhile, it is not conducive to exclude the interference of impurities in environmental water samples. Therefore, the best sample purge temperature is set at 60°C.

3.4 Salting Out Effect

To increase ionic strength, strong electrolyte (sodium chloride) was added into the solution. The "salt effect" to

reduce the solubility of compounds in solution, thereby contributing to the volatilization of the compounds^[15]. The effect of salt content on the extraction of odorous compounds was obtained (Figure 5.). The peak area between 0% and 5% vary greatly, indicating the significance of salting out effect. At the same time, a rising trend of peak area can be seen between salinity from 0% to 25%, but, from 20% to 25%, it has a little increase. Considering that the excessive salt will lead to precipitation, making it easy to plug equipment, and not benefit for the long-term operation and maintenance of it. Therefore, 20% is the optimum salt concentration.

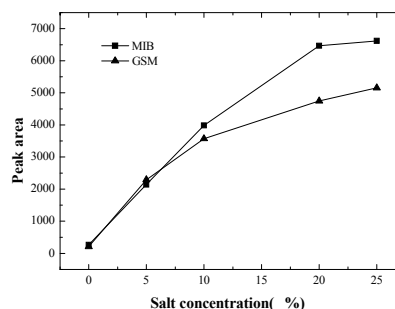


Figure 5. Salting out effect

3.5 Method Evaluation

The mixed standard (1mg/L) of MIB and GSM is dilution into 5, 10, 50, 100, 150, 200, 500ng/L, by adding internal standard (500µg/L) 25µL. A linear relationship was obtained for MIB and GSM and the correlation coefficients were 0.9998 and 0.9999, respectively. Meanwhile, The accuracy and precision of this method under three different lever of concentration (20ng/L, 100ng/L and 500ng/L) are measured in a reservoir samples matrix (Table 1.).

As can be seen from Table 1., a good linearity is gained in the range of 5-500ng/L. The detection limits of MIB is 4.12ng/L and 3.60ng/L for GSM, which are much lower than the limits of them in the "standards for drinking water quality" (GB5749-2006) in China. The results show good recoveries (81–111%), indicating acceptable accuracy at different concentrations levers. The precision of the method was also very good, showing low relative standard deviations (RSD) (ranging from 0.64–11.10%). Therefore, this trace determination method of the typical odorous compounds shows the advantage of high accuracy and precision; short analysis time and good stability.

3.6 Application to the Analysis of Environmental Water Samples

The collected water samples were transported at 4°C. Sodium sulfite was used for dechlorination and determined within 14 days. Water samples should filter through 0.45 µm membrane before determined^[16]. The

results are shown in Table 2.

From Table 2., we can see that this method is suitable for determination of different matrix water samples, and GSM in all of the measured water samples are sig-

nificantly higher than MIB, which are consistent with the literature reported^[17]. It may be due to the number of algae or bacteria which produce GSM much more than MIB.

Table 1. the Method evaluation Indicators

Compound	RT (min)	Selected ions	r	LOD	Matrix spike concentration (ng/L)					
					20		100		500	
					Recovery(%)	RSD(%)	Recovery(%)	RSD(%)	Recovery(%)	RSD(%)
GSM	20.933	112	0.9999	3.60	81-104	11.10	82-95	5.20	97-98	0.64
MIB	14.788	107	0.9998	4.12	89-111	8.86	99-101	0.78	102-108	2.62

Table 2. The GSM and MIB content of environmental water samples

Compound	Reservoir source water	Lyngbya kuetzingii culture	Samples		
			sewage pond Water	Park Landscape Water	Pond Water
MIB (ng/L)	ND	ND	ND	ND	ND
GSM (ng/L)	7.37	1.26×10 ³	418.59	30.67	31.34

The detection limit of GSM and MIB are 3.60ng/ L and 4.12ng/ L.

4 Conclusion

In this paper, The determination method of geosmin and 2-methylisoborneol using isotope dilution purge-and-trap gas chromatography mass spectrometry was set up. This method have the following advantages. 1. All the analytes will be almost purged out and can concentrated, which provide the high extraction efficiency; 2. No organic solvent pre-treatment method will not contribute secondary pollution to the environment; 3. Less sampling volume subjects to smaller matrix interference and is easy to actualize on-line inspection; 4. The isotope dilution technique guarantees the quantification precision. To sum up, This method is not only simple, but also fast, accurate, high precision, it is satisfied for the determination requirement of GSM and MIB in the “standards for drinking water quality” (GB5749 - 2006) in China.

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