Study of the presence of geosmin in bottled mineral water by CLSA and GC-MS

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Estudio de la presencia de geosmina en agua mineral embotellada por CLSA y GC-MS

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RESUMEN

El consumidor evalúa la calidad del agua a través de sus propiedades organolépticas, de entre las cuales el gusto y el olor son las más importantes. La geosmina es uno de los compuestos que ha generado más episodios de gusto y olor en el agua de bebida aportando un olor descrito como “tierra mojada”. En este estudio se han analizado muestras de agua embotellada que presentaban el citado mal olor y se ha detectado la presencia de geosmina en concentraciones por encima de su umbral de percepción. La extracción y concentración de la geosmina se realizó mediante CLSA (*closed loop stripping analysis*) mientras que su detección y cuantificación se llevaron a cabo mediante cromatografía de gases acoplada a espectrometría de masas (GC-MS). Se han descrito numerosos casos donde se ha detectado la presencia de la geosmina, incluso en agua potable, pero según la bibliografía consultada, este es el primer estudio en que se detecta en agua mineral embotellada.

Palabras clave: agua embotellada, CLSA, GC-MS, geosmina, tierra mojada

SUMMARY

Organoleptic properties of water are the only means whereby a consumer can assess water quality and safety. Among these properties, taste and smell are the most important. Geosmin is a compound whose presence has been causing a large number of episodes about taste and smell in drinking water. Its smell has been described as “earthy-musty”. In this study several samples of bottled mineral water having an “earthy-musty” off-flavor have been analysed and concentrations of geosmin above its perception threshold have been detected. Closed Loop Stripping Analysis (CLSA) has been used as extraction and concentration method. Detection and quantitation of geosmin have been carried out by gas chromatography-mass spectrometry (GC-MS). Many studies have reported the presence of geosmin in water and drinking water, but to our knowledge, this study is the first one where geosmin has been detected in bottled mineral water.

Keywords: bottled mineral water, CLSA, earthy-musty, GC-MS, geosmin,

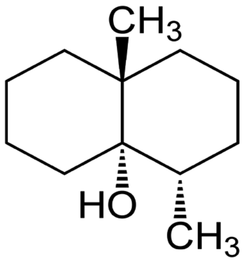
RESUM

Les propietats organolèptiques de l’aigua són la única via per la que un consumidor pot valorar la seva qualitat i seguretat. De les esmentades propietats, el gust i la olor són les més importants. La geosmina és un dels compostos que ha generat més episodis de gust i olor en l’aigua de beguda aportant una olor descrita com de “terra mullada”. En aquest estudi s’han analitzat mostres d’aigua envasada que presentaven aquesta mala olor i s’ha detectat la presència de la geosmina en concentracions superiors al seu llindar de percepció. Com a mètode d’extracció i concentració de la geosmina s’ha utilitzat el CLSA (*closed loop striping analysis*) i per a la seva detecció i quantificació s’ha utilitzat la cromatografia de gasos acoblada a l’espectrometria de masses. S’han descrit nombrosos casos on s’ha detectat la presència de geosmina en aigua, fins i tot en aigua potable, però segons la bibliografia consultada, és el primer cop que es detecta en aigua mineral envasada.

Paraules clau: aigua mineral embotellada, CLSA, GC-MS, geosmina, terra mullada

INTRODUCTION

Taste and smell are the organoleptic properties most appreciated by water consumers[1,2]. Frequently, tap water presents no harmlessness and insipidity as it should expected, due to the chemical treatment to which water has been submitted, although their safety is guaranteed owing to the rigorous controls applied. These unwanted taste and/or smell, along with the popular belief that bottled water provides health benefits[3], have made that the sales of this kind of water have increased during the last decades[4].
The legislation that regulates bottled water in Spain is set out in RD 1798/2010 adapting European directives 2009/54/EC and 98/83/EC. Under this decree, mineral water is defined as water from underground sources, and microbiologically healthy. No treatment able to change the water natural composition can be applied to a mineral water. The only allowed processes are filtration, oxygenation, settling and treatment with ozone-rich air.
Throughout this study, twelve samples with strange smell described as "earthy-musty" by consumers were analysed, and geosmin would be the compound capable of generating this kind of smell[5]
Geosmin is a semi-volatile organic compound (SVOC) with an alcoholic group and two cyclohexane groups sharing two carbons as shown in Figure 1. It was discovered by Gerber and Lechevalier in 1965[6], and the detection threshold for the natural enantiomer (the (-) isomer) is 9.5 ng/L[7].



There are several methods to extract the volatile and semi-volatile organic compounds from water; among others, the following ones can be highlighted: purge and trap (P&T), simultaneous distillation and extraction (SDE, aka Likens-Nickerson method), liquid-liquid extraction (LLE), solid phase micro extraction (SPME), closed loop stripping analysis (CLSA) and stir bar sorptive extraction (SBSE). CLSA is one of the most effective and versatile methods to extract and concentrate the compounds responsible for providing water with taste and smell. This method yields high recoveries and high concentration factors, the above qualities justify the inclusion in Standard Methods[8]. After the SVOC have been extracted and concentrated, they are separated, identified and quantified by gas chromatography coupled to a mass spectrometry detector (GC-MS), and whose detection limits are shown below ng/L (ppt).
The presence of geosmin in water has been widely studied from groundwater [9], lakes[10–14], waste waters[15], supply systems[16,17] and tap water[16,18]. To our knowledge, this study would be the first one detecting the presence of geosmin in bottled drinking water.
The aim of this work is the identification and quantitation of geosmin, responsible for generating the "earthy-musty" odor in bottled mineral water by applying CLSA and GC-MS techniques.

MATERIALS AND METHODS

Samples of bottled mineral water
The samples provided by bottled mineral water companies –all of them subject of complain about their off-flavour– were stored at 4ºC until they were analysed. The bottles were polyethylene terephthalate (PET) containers with polyethylene (PE) caps. The volumes of the bottles were 0.33, 0.5 and 1.5 litres.
Standards and solvents
The standards of geosmin and 1-chloroalkanes (C_5, C_6, C_8 C_{10}, C_{12}, C_{14}, C_{16} and C_{18}) were supplied by Sigma-Aldrich (St. Louis, Missouri, USA). The 1-chloroalkane standards used in the control of CLSA were diluted in CS_2 (Fluka, Buchs, Switzerland). The geosmin standards to validate the method were diluted in acetone (Burdick & Jackson, Seelze, Germany).
Extraction and concentration of the samples by CLSA
Extraction and concentration of VOCs and SVOCs were carried out using a CLSA 9000 equipment (Brechbüler, Switzerland) following the method (with some modification) developed by Grob[19] and standardized by Krasner *et al.*[20]. The procedure for analysing the water samples was as follows: 10 µL of 1-chloroalkane standards (40 ng/L) were added to 950 mL of bottled water in a 1 L bottle for CLSA extraction and placed in a thermostatic bath at 45ºC for 60 minutes. VOCs and SVOCs were swept away to the activated charcoal filter by an air flow of 4.2 L/min. The function of the filter was to retain the VOCs and SVOCs present in the water sample. The following step was the desorption of the activated charcoal filter, carried out as follows: recovery standards were added to a 20 µL vial and the compounds retained were eluted by $SC_{2.}$ This procedure allows concentrating the compounds present in 950 mL of water in a volume of 20 µL. If the sample volume was less than 950 mL, controlled mineral water was added until reaching the volume needed
Separation, identification and quantitation by GC-MS
The extracts obtained from CLSA method were analysed by GC-MS (Trace MS Plus, Thermo Finnigan, San José, California). Helium was the carrier gas, its flow rate was 1 mL/min. The separation of compounds was performed on a DB-5 capillary column (J&W, Agilent, Santa Clara, California) (60 m x 0.25 mm ID x 0.25 µm film thickness). Column temperature was held at 35ºC for 5 min and increased to 280ºC at 5ºC/min, holding 10 min. 1µL of aliquots were injected in cool on-column mode. The temperatures of ion-source and the transfer line were respectively 175 and 280ºC. Electron impact mass spectra were recorded at 70 eV ionization energy. The GC-MS analysis was carried out both in full scan mode (from m/z 35 to 200, 0.5 s/scan) and in selected ion monitoring (SIM) to respectively determine their presence and quantitation. Selected ions for SIM were 112 and 126 with dwell time of 60 ms.
Geosmin was identified by comparing their mass spectrum with that of the mass spectrum libraries Wiley 6.1 (New York, USA) and Nist05 (Gaithersburg, Maryland, USA) and comparing its mass spectrum and retention time with those of the corresponding standard compound.
Quality parameters
In order to evaluate the performance of the method for the analysis of water samples, linearity, instrumental detection limit (IDL), instrumental quantification limit (IQL), repeatability, reproducibility and recovery in bottled water were

calculated. These quantitation and detection parameters are shown in table 1.
The linearity range was evaluated by plotting the calibration curves of the area relative to the closest internal standard (chloroalkane C_{12}) *versus* the concentration of geosmin at seven levels straight line, which were injected in triplicate. Correlation factors (r^2) were obtained from these data. Linearity covers a range from 0.2 to 40 ng/L and it has a correlation coefficient r^2 greater than 0.999.
IDL and IQL were calculated by injecting in triplicate the less concentrated standard and by applying a signal to noise ratio of, respectively, 3 and 10. The detection and quantitation limits injected in the GC-MS are 0.25 pg and 4.9 pg, corresponding respectively to 0.005 ng/L and 0.1 ng/L in sample water. These low levels allow detecting geosmin in all samples because they are below the odour and taste previously published detection thresholds.
Repeatability was calculated by injecting four concentration levels five times during the same day and the reproducibility was calculated by injecting the same level concentration levels during different days. Repeatability has very low values, being 2% the highest value corresponding to a concentration of 50 pg/L. The values of reproducibility increase slightly without exceeding 10%. Taking into account that the on-column injections are manual, the results obtained can be considered satisfactory.
Geosmin recovery was calculated by adding 200 ng/L of standard to 950 mL of water and then the extraction was carried out the same way that the samples previously analysed. This procedure was repeated three times. The recovery obtained is similar to those of other studies (88%)[21].

Table 1. *Quality parameters of geosmin.*

Parameters	
Linearity range (ng/L)	0.2-40
Correlation coefficient r^2	0.9997
IDL (pg)	0.25
IQL (pg)	4.9
Repeatability (%RSD)	
50 pg/L	2.0
0.2ng/L	0.3
4 ng/L	0.8
8 ng/L	0.2
Reproducibility (%RSD)	
50 pg/L	9.8
0.2ng/L	9.5
4 ng/L	6.1
8 ng/L	8.8
Recovery (%)	88

RESULTS AND DISCUSSION

CLSA is a method that allows detecting a wide range of VOCs and SVOCs present in water, including whose provide those possessing an "earthy-musty" smell[21,22]. According to the classical smell water wheels[23], the compounds generating this kind of smell are MIB (2-methylisoborneol), geosmin, several trichloroanisoles and pyrazines.

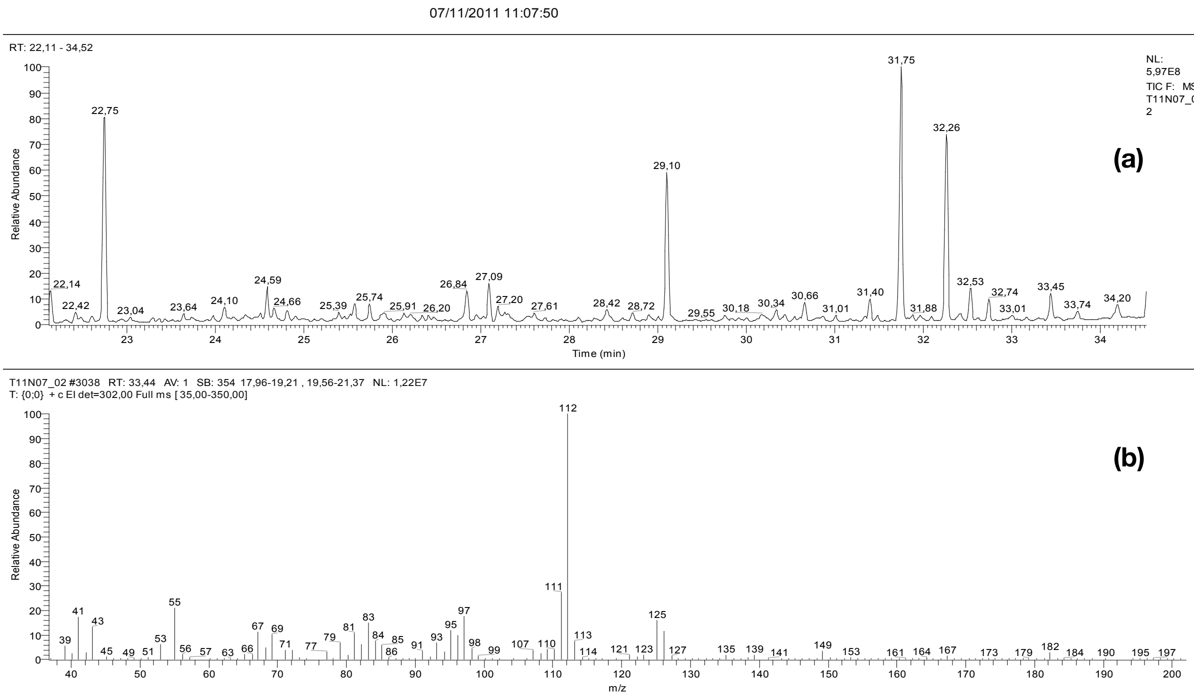
Among the twelve samples analysed that smelled like "eathy-musty" according to the consumers, geomsin was detected in four of them, as shown in table 2.

Table 2*. Concentration (ng/L) of geosmin detected in samples of bottled water.*

SAMPLE	GEOSMIN
SAMPLE 1	2 ng/L
SAMPLE 2	7 ng/L
SAMPLE 3	28 ng/L
SAMPLE 4	30 ng/L

Two samples had a geosmin concentration below 10 ng/L and the other two had a concentration about 30 ng/L. Although the concentrations are very low, the odour and taste detection in water at 40ºC is 3.8 ng/L and 1.3 ng/L, respectively[24]. In addition, they are above the detection and quantitation limits. Figure shows the chromatogram (TIC) and spectrum of geosmin obtained from one of the samples.
Although consumers tend to associate taste and smell as indicator of water safety, the concentration of geosmin in the bottles studied has not been associated with any health effect[25]. Geosmin have also not been correlated to presence of cyanobacterial toxins which are extremely toxic. As a result, there is no maximum contaminant level (MCL) for geosmin and there are currently no regulations[26].
The geosmin detected in this study can come from different origins like the source, contamination during the bottling stage, wet storage area, poor quality of packaging and time out on the market[27–29]. Concerning the source, the primary responsible for the presence of geosmin and/or MIB in water are cyanobacteria (aka blue-green algae)[10,12]. In addition, other studies have shown the presence of geosmin and/or MIB on the surface of water and this is due to the certain type of filamentous bacteria or actinomycetes[30]. Cyanobacteria synthesise MIB or geosmin during their growth and can release these odorants depending on the growth phase according to several environmental factors, mostly during the death and biodegradation of these cells[26]. The process more effective to remove geosmin from water are activated carbon adsorption or oxidation technologies such as ozone or UV with H_2O_2[26].The Spanish rule on bottled mineral water does not allow these processes, but prepared waters can be subjected to any treatment that modifies the original water in order to comply with chemical, microbiological and radiological safety requirements for pre-packaged water, and its legislation is set out in RD 1799/2010. In reference to consumers, and to avoid these problems, consumption date should be respected. In addition, the place and storage conditions should be taken into account.
On the other hand, any accidental contamination (bottling stage, packaging and storage) requires better knowledge to understand the origins and causes. In all cases, a reliable method for detection and quantitation of geosmin should be carried out in order to solve the drawback caused by the presence of geosmin in bottled mineral water.

Figure 2. *Total ion chromatogram (TIC) from GC-MS analysis of bottled mineral water (a) and mass spectrum of geosmin (b) detected in a real sample.*



CONCLUSIONS

- The CLSA method along with a GC-MS method has been developed to detect geosmin at very low concentrations. The amount of extract obtained with each extraction allows several analyses from the same extract.
- To our knowledge, geosmin has been detected for the first time in bottled mineral water.
- The geosmin concentrations detected range from 2 to 30 ng/L. Despite there are no regulations for geosmin, these concentrations do not involve any health risk.
- Further studies would be required in order to better understand the sources and fate of geosmin in the bottling process, storage, the microbiology of water and the geochemistry of the undergrounds sources, in order to prevent the presence of compounds capable of altering the organoleptic quality of water, like geosmin.

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