Chromatography Technical Note No AS179
A Fully Automated Liquid-Liquid Extraction for 2-Methylisoborneol and Geosmin in water

Richard Davis, Kathy Ridgeway, Jonathan Dunscombe *Anatune Ltd. Girton, Cambridgeshire (UK)*

Introduction

2-methylisoborneol (MIB) and geosmin are problematic compounds in the water industry. Cyanobacteria in algae and actinomycetes in bacteria produce these compounds naturally in surface water sources, which, if not removed can be detected in drinking water. Humans are able to detect the musty/earthy odour of these compounds at extremely low levels, 5ng/L for MIB and 30ng/L for geosmin respectively. Water testing methodologies therefore require equally low detection limits to identify these compounds and treat the water.

Often manual sample preparation methods, such as liquid-liquid extraction (LLE) can be time consuming and require large volumes of sample and solvents. Rapid turnaround of samples is very important in the industry to enable responsive treatment by water companies.

Anatune recently developed a completely automated solution, using the QuickMix and CF-200 centrifuge options which miniaturized and speeds up sample turnaround.

Instrumentation

Dual Head GERSTEL MPS xt
GERSTEL Cooled Injection System (*CIS*) *4 PTV inlet*
GERSTEL QuickMix
Maestro software integrated
Agilent 5977B High efficiency source
Agilent GC 7890B
Anatune CF-200 Centrifuge
Masshunter B.07.04

Method

As the only manual step, 6ml of sample was transferred to a 10 mL high recovery vial.

Fully automated sample preparation was carried out individually immediately prior to injection. The prep-ahead function of GERSTEL Maestro enabled each sample/standard to be prepared during the subsequent sample/standard GC acquisition time.

Calibration standards and samples were prepared in bottled water in the range of 2-100 ng/L by addition of stock solutions using the right hand head of the Dual Head GERSTEL MPS equipped with a 10 µL syringe. Table 1 details the volumes added. Internal standard was then subsequently added to each calibration standard/ raw water samples, by using the same syringe once it was cleaned.

Final Conc [ng/L]	Spike Conc [µg/L]	Vol (mL)	Vol spike (µL)
2.0	5.0	6.0	2.4
4.0	5.0	6.0	4.8
10.0	50.0	6.0	1.2
50.0	50.0	6.0	6
100.0	50.0	6.0	12

Table 1. Calibration spiking levels

Immediately after addition of the standards, 0.2 mL of dichloromethane was added to the vial and the vial transported to the QuickMix shaker and agitated at 3000rpm for 3 minutes. Each vial was then moved to the centrifuge CF-200 and centrifuged at 4500rpm for 5 minutes to remove any emulsions.

After centrifugation, the vial was transported back to the vial tray and 10 µL from the DCM solvent layer was injected directly in the CIS 4 inlet for large volume injection (LVI) using the right hand head.

The CIS inlet was operated in the solvent vent mode and ramped from 10°C to a final temperature of 280°C. Chromatography was performed on a DB-5MS UI column.

Results

Linearity and precision was carried out for both geosmin and MIB.

Figure 1 shows the linearity obtained for geosmin. Linearity is >0.999 with reference to internal standard. Attaining good linearity demonstrates the feasibility of using this equipment for the application.
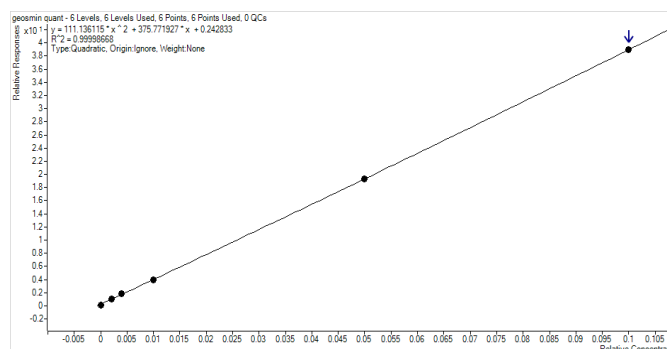

Fig 1 linearity of geosmin. $R^2 – 0.9997$.

Precision was carried out using a set of ten samples spiked at 2ng/L level which resulted in less than 10% RSD% for both geosmin and MIB. See Table 2.

Sample	MIB (ug/L)	Geosmin (ug/L)
1	0.0024	0.0025
2	0.0025	0.0023
3	0.0023	0.0025
4	0.0028	0.0025
5	0.0024	0.0024
6	0.0026	0.0022
7	0.0025	0.0025
8	0.0026	0.0026
9	0.0028	0.0026
10	0.0027	0.0025
Mean	0.00256	0.00246
SD	0.00017	0.00013
%rsd	8.56	6.32

Table 2 precision for geosmin and mib

Figure 3, shows a chromatogram of geosmin at 10ng/L. Some chromatographic noise is evident, however resolution is sufficient to identify the peak. Use of qualifiers and masshunter integration software would ensure correct identification of the peak.
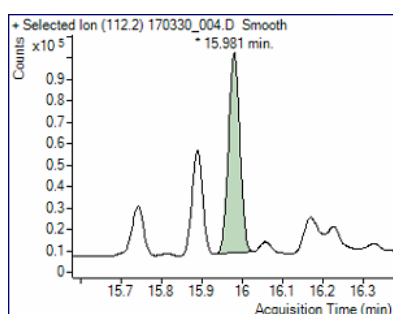

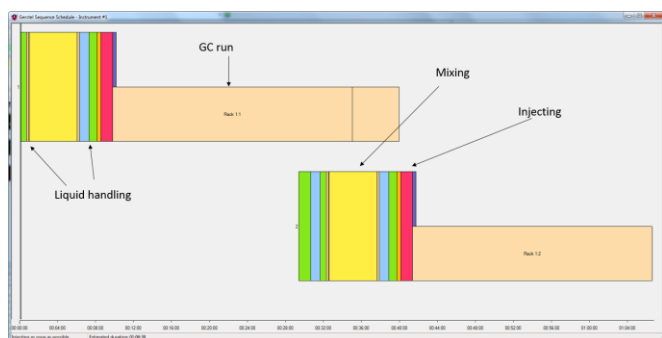*Fig 3 chromatogram of geosmin quant m/z 112*

Fig 4 prepsequence and prepahead.

The GC run time was 25 minutes giving a total of 40 mins for the sample prep and GC run. Fig 4 shows the prep sequence used for this procedure. In the diagram above, each coloured block represents a different action that occurs during the whole sample preparation and analysis sequence – the multi-coloured part being those relating to the extraction – addition of standards, solvents, mixing and centrifugation. The light brown is depicting the GC run. Using the prepahead function, the software has been able to arrange the process so that extraction of one sample takes place whilst another extract is being analysed. Once the first sample has been processed, this can save 15 minutes per sample. If compared to a manual method

where the samples would be extracted and then analysed on GC, with a typical extraction time of 45 minutes and analysis time of 20 minutes, it is clear how this function can enable the laboratory to drastically increase sample throughputs and divert analyst time to perform other duties much more needed. This approach also allows sample preparation to occur without staff being physically present in the laboratory due to the ability of the whole sequence to be fully automated. Sample turnaround times can be drastically improved because of this.

This work was carried out using 10ml high recovery vials. As dichloromethane is denser than water, the use of these vials is a requirement to ensure accurate recovery of the solvent for injection.

Whilst these vials are more expensive than the standard 10ml vials used with the MPS, this cost is offset by reduction in use of solvents, transportation of 1 litre sample bottles and other consumables associated with manual preparation. In addition, further costs per sample savings have the potential to be obtained by reducing the number of repeat analysis batches due to errors from manual sample preparation Figure 5 shows the difference in solvent use between the manual and automated method.
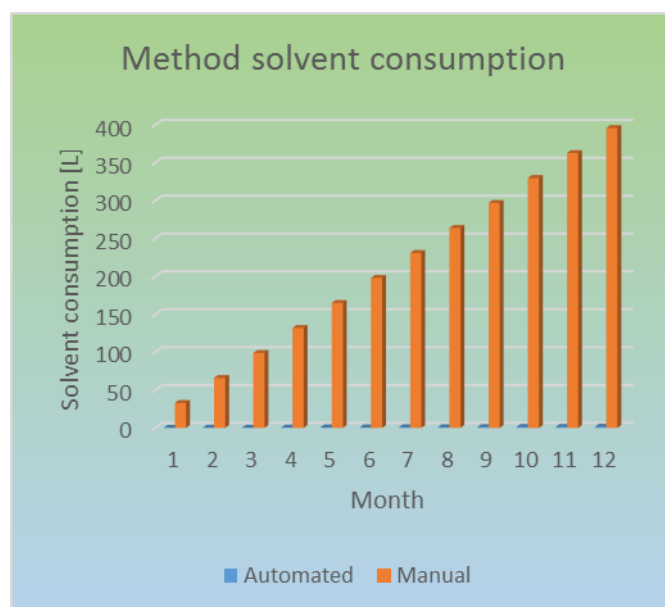

Fig 5 Solvent consumption over 12 months (based on 30 samples extracted per day for 260 days of the year).

These figures were based on preparing 30 samples a day for 260 days of the working year. An approximate value of 394 litres of solvent can be saved over the year. This would contribute to a significant saving of both solvent and in-lab costs. This saving in solvent also goes someway to environmental targets.

Discussion

This work shows how extraction of MIB and Geosmin compounds can be miniaturized and automated using the Gerstel MPS. This can be accomplished whilst achieving the required limits of detection needed, allowing the sample preparation to be carried out robotically. It also ties in with other previously released APP notes AS168, AS170 and AS171 demonstrating how a system with the same set up can be used for multiple methods.