The Automation of High Capacity, High Throughput, Static Headspace – GC-MS

Headspace sampling is an established technique for quantitation of volatile organic compounds in various types of samples. Since only gas phase material is injected into the GC-MS, static headspace sampling methods tend to be robust and reliable – this makes static headspace methods well suited to high throughput applications where large numbers of samples need to be analysed routinely. Careful optimisation of both chromatographic and mass spectrometer parameters enables very short cycle-times to be achieved, even with complex samples. For a laboratory to best exploit this capability, a high degree of automation is needed. This article details the development of an automated system for fast, high capacity, static headspace analysis that is capable of 24 hour unattended operation.

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INTRODUCTION

Laboratories face difficult challenges, especially during the current economic climate. The cost of consumables, employees' wages, capital equipment and utilities is rising, yet there is a significant pressure from clients for the price of sample analysis to remain low.

The impact of current and forthcoming legislation will also mean that many laboratories will experience a significant increase in the number of samples that need to be analysed.

For a laboratory facing increased sample throughput, there are choices that must be made. Increasing equipment and staff in proportion to the increase in sample numbers is rarely a viable option. It is usually far better to optimise methods for greater speed and to aim for full utilisation of instrumentation by increasing the degree of automation, keeping staff levels the same.

This philosophy was applied to the analysis of volatile organic compounds (VOCs) by static headspace and an effective, high throughput, high capacity solution was evolved.

The development began with three applications in mind – VOCs in waste water, VOCs in environmental soil samples and 'Red-List' solvents in drinking water. In each case, the analysis was a regulated method and so a clear set of performance specifications existed that the instrumental solution would have to satisfy.

An evolutionary approach was applied to the development of the VOC analyser and the development proceeded via a number of stages, each geared to removing the current bottle neck in the analytical process. Care was taken to ensure that upgrade paths were maintained so that at virtually any stage, existing customers could take advantage of recent enhancements to the capability of the system by upgrading their existing instrumentation to the current standard.

THE DEVELOPMENT PROCESS

The first stage of the development focused on minimising the GC-MS run time. It was not uncommon for laboratories to be operating VOC methods with cycle times (injection to injection) of 45 minutes per sample with the mass spectrometer operating in SIM mode for maximum sensitivity. A few points were apparent at the outset, firstly, setting and maintaining SIM windows would be become increasingly difficult with faster run time and so the decision was to work in scan mode. This presented the opportunity of utilising the greater range of quantitation ions, enabling chromatographic separations (and the associated run time penalties) to be minimised. This phase of the development resulted in the cycle time being reduced to 8 minutes for a target suite of 70 compounds. The loss in sensitivity due to operating is scan was (as anticipated) compensated for by the sharper peaks yielding a better signal to noise ratio.

The decrease in cycle time meant that the instruments potential sample throughput increased from approximately 32 samples per 24 hour period to around 180 samples per 24 hour period, a five-fold increase in sample throughput, making auto sampler capacity now the limiting factor in the number of samples that could conveniently be run per day.

The GERSTEL MPS Robotic Sampler had a maximum capacity of 96 x 20ml headspace vials, however, it was clear that a re-designed sample tray and tray-holder could accommodate far more samples. After several iterations of design, the current arrangement was settled on that permitted several tray capacities to be accommodated ranging from 160 to 240 vials. This allowed users to select a sample capacity that complemented the pattern of work in their individual laboratories. Laboratories that worked 8-hour shifts could now readily achieve 'round-the-clock' usage of their GC-MS for VOC analysis.

AUTO SPIKING

From discussions with users and from our own experience of running large sample batches, it was clear that the manual preparation of large numbers of samples prior to headspace analysis represented a significant amount of work. As such it was clear that big benefits could be gained from automating all or part of the sample preparation process.

A large part of this work comprised spiking samples with internal standards and surrogate compounds. This is time consuming, repetitive work that requires great care and concentration on behalf of the operator. It is relatively common for errors to be made resulting in invalid results and samples having to be re-analysed. One other consequence of the manual process was that the time elapsed between the addition of the standard and the sample being run varied a great deal across a large batch of samples. This often resulted in reported results exhibiting some degree of drift throughout the batch of samples.

It was decided to initiate an application development project to enable automated spiking of surrogates and samples and to perform this in a 'just-in-time' fashion, thereby ensuring that every sample in the batch would be treated the same. It was anticipated that this approach would improve consistency of results across large batches of samples.

The headspace autosampler used a heated 2.5 ml syringe to inject samples into the GC-MS - the automated addition of small volumes of liquid phase requires the use of a liquid syringe. In order to provide for this, a dual-rail GERSTEL MPS PrepStation was used for the task; this enabled two different syringes to be employed – one heated and one at room temperature.

The protocol that was settled on as a result of this work was as follows: for each sample the operator adds a quantity of sodium sulphate to a vial, pipettes an aliquot of sample into the vial, seals the sample and places the vial in the instrument's sample tray. The final manual task is for the operator to place a mixture of internal standard and surrogate solutions into several 2ml vials and load these into the internal standard position on the auto sampler tray.

The instrument begins by injecting the required volume of the internal standard mixture into the first sample vial, which is then placed into the heated agitator where the sample is incubated for a fixed time. Every subsequent sample is treated in exactly the same fashion.

Autospiking was demonstrated to be a practical proposition and gave results that demonstrated excellent repeatability. No significant drift was observed across a batch of 240 samples for any of the surrogate compounds.

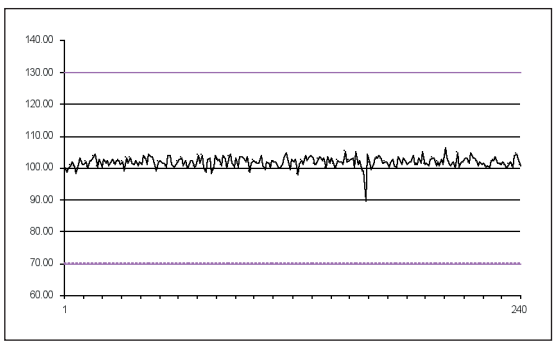

Figure 1. Shows the calculated concentration of the surrogate compound 1,2-Dichloroethane-d6 for each sample of a batch of 240 samples. This was spiked at a concentration of 100ng/l - the two horizontal lines represent the acceptable limits of 70-130µg/l.

RESULTS

The Anatune VOC analyser was initially aimed at three primary applications:

- Volatile organic compounds in waste water
- Volatile organic compounds in soil
- 'Red List' solvents in drinking water

The first two environmental applications feature a target compound list of some 70 compounds over a calibration range of 3-200µg/l. For the Red List solvents a smaller suite of compounds is involved with a calibration range of 0.2-20µg/l.

In all cases, the headspace analyser is used to add standard, internal standard and surrogate solutions automatically as part of the instrumental method.

A batch of water 240 samples was prepared by adding sodium sulphate to vials, water was subsequently added and then the vials were capped. The headspace analyser was then used to add internal standard and surrogate solutions to each vial in a 'just-in-time' manner prior to their headspace analysis.

The mean abundance and % RSD figures for each internal standard from the entire batch of 240 samples are listed below:

Internal Standard	Mean Abundance	% RSD
Pentafluorobenzene	28614331.37	9.91
Difluorobenzene	36178592.78	10.61
Chlorobenzene-d5	15734441.63	10.25
1,4-Dichlorobenzene-d4	18727709.07	9.83

The mean concentration, % RSD and bias figures for each surrogate from the entire batch of 240 samples spiked at a concentration of 100µg/L are listed below:

Surrogate Analytes	Mean Calculated Concentration (µg/L)	% RSD	Bias (%)
1,2-Dichloroethane-d6	101.78	1.62	+1.78
Toluene-d8	99.53	1.40	-0.47
Bromofluorobenzene	101.06	1.43	+1.06

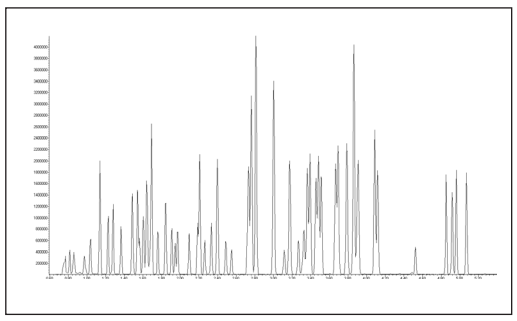

Figure 2: A chromatogram of a 200µg/l standard.

CONCLUSION

It has proved perfectly feasible to develop a practical solution for the fast analysis of samples containing complex mixtures of volatile organic compounds and to do so using somewhat conventional technology that results in a highly robust and reliable system.

A significant part of the cost of any analysis is the cost of the instrumentation used to make the measurement. One very effective way of mitigating this cost is by careful design, to maximise the number of samples that the instrument can run each day, thereby spreading the cost between more samples.

Further savings are made possible by automating as much of the analysis as possible.

Automating the spiking of volatile standards is technically challenging due to the ease with which evaporative losses can occur. However, despite this, it has been shown to be a practical proposition and can be used both to save skilled analyst's valuable time and to further reduce the opportunity for human error in the analytical process.

Characterising Hydrodynamic Changes in Polypeptides

Research carried out by scientists from the Institut Pasteur in Paris, France, has shown that size exclusion chromatography followed by triple detection array (SEC-TDA) can distinguish between oligomerisation, hydration and shape changes of proteins. The work was conducted using advanced GPC/SEC systems from **Viscotek**, a **Malvern Company**. Using a Viscotek Triple Detector Array (TDA302) system coupled to a GPCmax chromatographic system, the team characterised the structural and hydrodynamic properties of a fragment of the adenylate cyclase (CyaA) toxin, a major virulence factor of Bordetella pertussis, the causative bacteria of whooping cough. The research team demonstrated that calcium binding induces important hydrodynamic changes in the protein, gaining important insights into its biological function.

Conventional calibration of size exclusion chromatography (SEC) is based on known hydrodynamic volume of standard proteins. Such a calibration procedure suffers from the drawback of possible interactions between the protein of interest and the SEC matrix. Hence, conventional calibration can provide neither the molecular mass nor reliable hydrodynamic information. The Viscotek TDA302 uses a series of detectors to analyse the eluting sample, including: a UV-Visible spectrophotometer, a differential refractometer (RI), a 7° Low Angle Light Scattering (LALS), a 90° Right Angle Light Scattering (RALS) detector and a differential pressure (DP) Wheatstone bridge viscometer. OmniSEC software analyses all the collected data, presenting it in an information-rich format. The UV-detector and the RI are used to measure protein concentration, which is required to determine both the absolute molecular mass (MM) and the intrinsic viscosity (IV). MM is calculated directly from the light scattering data and IV from the viscometer. The IV results give insight into protein hydration and shape. In this application, IV is the only hydrodynamic parameter significantly affected by calcium binding. The information that the system supplies is therefore essential towards understanding protein binding and folding behaviours which can be key towards understanding how these molecules interact with the human body.

New High Performance Low Cost Ion Chromatograph

Metrohm present the 883 Basic IC plus, their new entry-level model to intelligent ion chromatography. The 883 Basic IC plus is the smallest ion chromatograph available in the market and has been designed to meet the requirements of schools and universities as well as routine applications in particular.

The 883 Basic IC plus features all the intelligent functions of Metrohm's high-end ion chromatographs. The iColumn technology facilitates the handling of columns and protects them against operational damage. Also included are the intelligent high-pressure pump iPump and the iDetector, Metrohm's intelligent conductivity detector. Both components guarantee precise results and virtually eliminate operator errors. Moreover, the 883 Basic IC plus is equipped with Metrohm's «MSM II» Suppressor Module for chemical suppression.

Operation of the 883 can be completely automated. For this, Metrohm offers the 883 Basic IC plus Package, which contains the 863 Compact IC Autosampler as well as the 883 Basic IC plus. This package is tailor-made for routine work and contains everything need for the automation of routine analyses. The 883 Basic IC plus is controlled by the MagIC Net™ Basic software, making operation of the system perfectly easy.