the Analytical Scientist®

SPECIAL SERIES: *GC–MS*

UPFRONT

Message in an Ancient Bottle

GC-MS uncovers the mystery contents of Bronze Age ceramic bottles

Gas chromatography-mass spectrometry (GC-MS) analysis of organic residues in ceramic bottles appear to have revealed an ancient trade in scented oils as long ago as the 3rd millennium BCE (1). The ceramic bottles, excavated from an archaeological site in Turkey, were suspected to have contained liquid, but no researchers had analyzed the residues inside them – until now!

GC-MS identified compounds in the bottles that suggest scented oils, providing the oldest evidence for trade in such commodities in the region. To learn more about the findings – which highlight the importance of GC-MS in archaeological investigations – we spoke with the study's lead author, Ismail Tarhan, Associate Professor of Biochemistry at Selçuk University, Turkey.

What was the inspiration for this work?

Archaeology has always been fascinating to me, and my interest was further fueled by a meeting with an archaeologist around five years ago. The thought of continuing my professional chemistry career in the field of archaeology was very exciting – so I started archaeometric studies, examining archaeological artifacts with science.

To begin with, I focused on the production conditions of open-mouthed ceramic artifacts, before intensifying my research to find an answer to a burning question: what was consumed or stored in these containers? Through trial and error of various analytical techniques, I managed to detect the degradation of food products within the containers.

What method was used for the analysis?

Ceramic artifacts have a mostly porous structure – allowing organic biomolecules to stay preserved inside. We analyzed the invisible micro-compounds in the inner walls of the ceramic and extracted the organic residues remaining in the pores of the ceramic structure into a liquid solvent. By putting this liquid through our GC-MS device, we detected organic residue molecules that showed what could have been held within each ceramic container.

What were your findings?

We discovered the presence of various traces of liquids from within each ceramic container, alongside dicarboxylic and oleic acids with large samples of palmitic acids. These substances suggest that a plant-based oil was contained frequently in these bottles. Furthermore, the presence of diterpenoids suggests the addition of ingredients such as conifer resin and other plant derived products. This leads us to conclude that the containers were used for scented oils.

Overall, we can determine that scented oils were exchanged in Anatolia, Turkey during the late 3rd millennium BCE. The different organic residues also indicate that a range of different recipes were used and contained within the containers for an undisclosed period of time.

Were you surprised by the results of your study?

We were surprised to learn that scented oils were highly prominent in the analyzed ceramics. We knew from archeological data that there was a high probability of finding traces of valuable herbal products, as opposed to products in daily consumption. However, it was a pleasant surprise to find similar results from organic residue analysis.

Are there any plans for future research?

There are many studies we would like to conduct as an extension of this research. Firstly, we plan to consolidate the results of GC-MS analysis with the results of stable isotope analysis alongside colleagues in Europe. Additionally, we've received many offers since publishing our study to conduct similar research with different excavations. We hope that exploring these avenues allows for more comprehensive analysis into our ancestors' livelihoods.

Reference

1. *I Tarhan et al., Toward an understanding of the exchange in ancient scented oils through organic residue analysis of Bronze Age Near Eastern ceramic bottles by GC–MS (2023). DOI: 10.1111/arcm.12852.*

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SPONSORED CONTENT

New in GC-MS: The Pegasus BTX

Introducing the Pegasus BTX, the latest generation of LECO's Benchtop (or BT) GC-TOF-MS series. For the past eight years, the Pegasus BT series has proven reliability in labs performing unbiased, non-targeted analyses and tackling difficult matrices. A diverse spectrum of investigators – including those in industries such as Petroleum, Life Sciences, Environmental, Pharmaceutical, Food Safety – have gained deeper insights into the complex chemistries of their samples thanks to the incredible Pegasus BT.

The Pegasus BTX retains the innovative StayClean® ion source, blinding fast full-spectrum acquisition rates, and 5 orders of linear dynamic range – all seamlessly orchestrated by ChromaTOF® software. A completely reimagined ion path and detector design delivers femtogram-level sensitivity in a miniscule footprint – analytes that previously eluded even advanced targeted GC-MS systems are unveiled with rich mass spectra.

At LECO, technological innovations never jeopardize our commitment to instrument quality and robustness: that's why all new BTX systems include a two-year detector warranty, as a testament to LECO's investments in ion path integrity.

This innovative instrument for GC and GCxGC applications marks a new chapter for LECO customers in the world of Separation Science.

The Lucky Lab is…

In 2021, LECO began offering scientists and chemists located in the US a chance to obtain a GCxGC-enabled Pegasus TOFMS for their laboratory.

This year, after reviewing hundreds of entries from hopeful labs, LECO selected Kate Perrault Uptmor as the recipient of a new LECO Pegasus BTX 4D with Paradigm™ Flow Modulator.

"LECO's Pegasus BTX 4D with Paradigm Flow Modulator is a great fit for the kind of work Dr. Perrault Uptmor is doing in her lab," said John Hayes, Separation Science Product Manager, in a press release. "Based on the lab's needs, the importance of the work being done, and the genuine passion for GCxGC, we believe she is the most deserving of this state-of-the-art equipment".

Last year's winner was Petr Vozka, Assistant Professor in the Department of Chemistry and Biochemistry at California State University. Dr. Vozka was chosen for his elaborate work in using GCxGC to study microplastics as well as his dedication to training the next generation of GCxGC users.

"The goal of the program is to not only empower labs that are doing truly amazing work with innovative tools, but also to partner with them in an effort to push the boundaries of innovation in solving complex chemistry problems using LECO's instrumentation and software," said Farai Rukunda, Director of Separation Science Customer Success.

IN MY VIEW

Mastering the Craft of Chromatography

Innovation in separation science continues apace, but a lack of skilled personnel with an understanding of the fundamentals will hold the field back

By David McCalley

Though chromatography has come a long way since I started my career, there are various difficulties that hinder the development of the subject. As the field stands at the beginning of 2024, the biggest challenge seems to be a lack of personnel who really understand the principles behind the techniques. Many can be classified as "application scientists" who follow set methods and are more interested in the results than studying the techniques themselves. I think the effects of serious skill shortages in the fundamentals of the subject have been avoided so far because a vigorous handful of academics and industrialists have been able to keep the research and development flag flying. But it's clear that the number of skilled personnel decreases year by year as they retire and are not replaced. Some are made redundant by outsourcing in industry. Outsourcing may have short term financial benefits to an organization, but once important skills are lost, a downward spiral can take effect.

In the UK where I am based, some recruitment from continental Europe or further afield has partially plugged the skills gap, but unfortunately other countries seem to have similar problems with the availability of people who are accomplished in the fundamentals. Research and development, even on a modest scale, are vital to the health of a subject area – not only to push forward the boundaries, but also in broadening experience by research-informed teaching and learning.

Despite challenging times, there are various exciting trends emerging in the field. Take the development of pillar array columns by Gert Desmet and colleagues in Brussels; higher column efficiency can be obtained with a regular structure of pillar arrays, which avoids many of the problems associated with preparation, packing, and performance of conventional columns. This is interesting stuff – there's plenty more to be discovered and challenges to solve; for example, problems occur when increasing the diameter of these new columns to match those commonly found in particulate columns.

Multidimensional chromatography – and its promise of increasingly higher resolution of complex samples – offers another vigorous area of research. But again, further development and successful implementation of this technology will depend on the availability – or training – of highly skilled personnel. It is possible that candidates for research training in chromatography with qualifications in disciplines other than pure chemistry may provide a larger recruitment pool. Many such personnel with a background in pharma, clinical, environmental, or forensic science already work with chromatography applications, but could be encouraged to take up more fundamental studies.

Perhaps if we – as a collective – exude sufficient infectious enthusiasm, we can use these methods to attract new talent. I hope so. We have not solved all the problems in chromatography, even though it is a "mature" technique – nor have we met all the needs of society. But therein lies the beauty of analytical science: exciting discoveries and much-needed progress are there for the next generation of chromatographers to make. Especially those who are ready, willing and able to embrace the fundamentals of the subject!

David McCalley is Professor, Bioanalytical Science University of the West of England, Bristol, UK

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APPLICATION NOTE

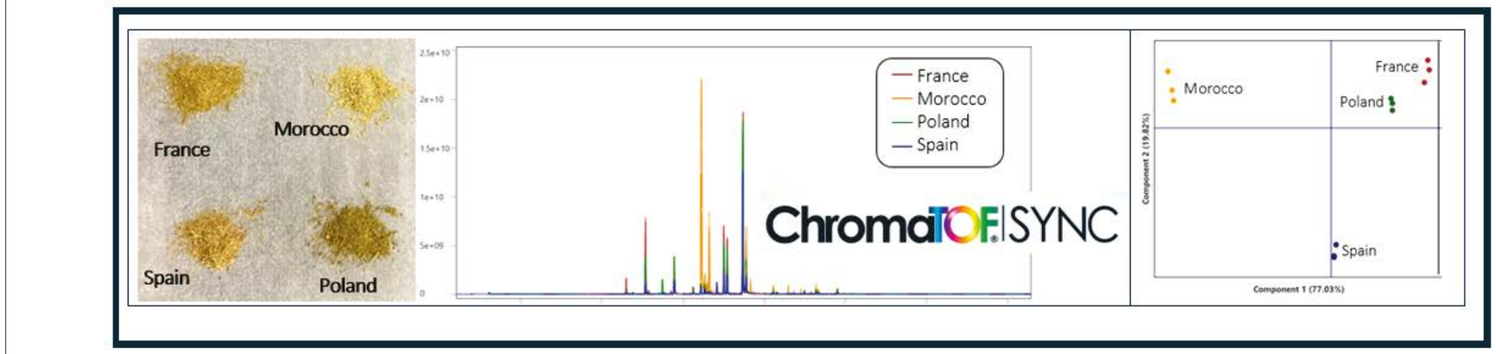
Enhanced Characterization and Comparison of Thyme Varieties from Different Geographical Origins

An analytical workflow solution combining GC-TOFMS and automated statistical data processing

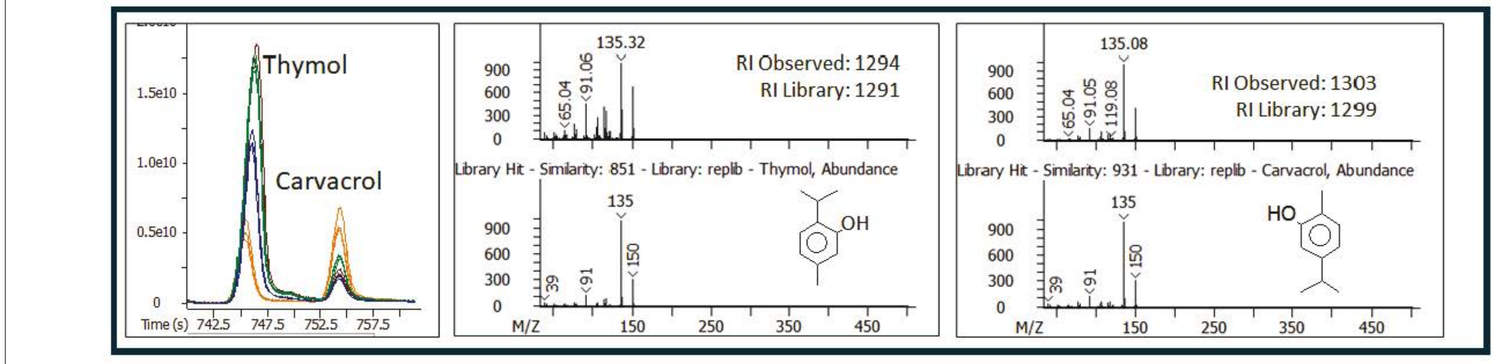
The aroma profiles of various herbs, spices, and other natural products are imperative in the flavor, food, and fragrance industries. Understanding the chemical profiles of these products is crucial in identifying how these chemicals may impact their aroma. Identifying the chemical compounds of Thyme and its origins allows businesses to respond quickly to consumer and market demands, leading to more efficient problem-solving and better outcomes.

In this study, the Pegasus BTX GC-TOFMS system and ChromaTOF Sync were utilized to assess and contrast dried thyme samples. Comprehensive non-target data, acquired with a full mass range and heightened sensitivity, was gathered, enabling efficient deconvolution and comprehensive coverage of analyte detection within these intricate samples. ChromaTOF Sync the statistical alignment and differentiation of analytes across the sample set, streamlining the comparison and interpretation process. Numerous unique analytes with captivating aroma profiles were identified in each thyme sample, and the alignment process allowed a fast and efficient highlighting of distinguishing chemicals between the four thyme origins to be performed.

SEE WHAT'S NEW WITH OUR THYME APP NOTE



The Pegasus BTX and ChromaTOF Sync are combined to compare dried thyme from four different countries.



Thymol and carvacrol are identified with library searching of the full m/z data and supported with RI matching to library.

FEATURE

DBPs: an Underestimated Threat

Why Susan Richardson's recent discoveries about disinfection by-products are (even more) cause for concern

Back in 2019, Susan Richardson, Arthur Sease Williams Professor, Department of Chemistry & Biochemistry, University of South Carolina, USA, wrote a feature for The Analytical Scientist discussing her work on disinfection by-products (DBPs) – and the threat they pose to our health, wildlife, and environment.

We recently connected with Susan to see where this research has taken her over the past few years, what she's mostly concerned about today, and how environmental analysis can progress in the near future.

What is the biggest analytical challenge in environmental research today – and how do you believe we can overcome it?

Identifying unknown contaminants, whether in drinking water or environmental surface waters, remains as our biggest environmental analytical challenge. It is much harder to identify new contaminants than those we already understand, and they are more likely to be found at very low levels in a sea of contaminants – it's like looking for a needle in a haystack!

Effect-directed analysis (EDA) is a valuable method for pinpointing harmful contaminants causing toxicity effects. However, it takes a great deal of time and effort, which discourages many groups from using it. There are several examples of EDA's effectiveness, such as the discovery of 6ppd-quinone harming coho salmon in the Northwest US (1) and a new algal toxin affecting bald eagles in the Southeast US (2).

Which environmental contaminant are you most concerned about today?

Despite having worked in various now-mainstream areas of environmental analysis, from microplastics to PFAS, I'm still most concerned about DBPs. This is because they're typically found at ppb levels (1,000 times higher than PFAS) and a body of evidence states that they cause serious effects to human health – such as bladder cancer, miscarriage, and birth defects. For example, dibromoacetonitrile – one of the emerging, unregulated DBPs – is carcinogenic in two animal species and often seen in drinking water at ppb levels (3).

What updates can you share about your work with DBPs?

In 2022, we published our new discovery on an important class of DBPs that weren't known before: halocyclopentadienes. We found these DBPs in real chlorinated and chloraminated drinking water using a very sensitive GC-MS instrument (4). I was especially surprised by two aspects of this research. Firstly, this class is the first to be bioaccumulative; secondly, hexachlorocyclopentadiene is now the most cytotoxic DBP known. These six new DBPs were found completely by accident by a PhD student in my lab, Jiafu Li, who spotted the new peaks and figured out what they were. As always with analytical science, important discoveries seem to appear when you least expect them!

Additionally, in a recent Environmental Science & Technology publication, we applied EDA to assess different size fractions of DBPs and determine which are most important toxicologically (5). We discovered that DBPs over 5000 Da molecular weight are not toxic – validating a statement made by a prominent toxicologist at a conference I attended. The most toxic fraction was <1000 Da, challenging the common focus on larger fractions in previous research.

How important is mass spec to your work?

Hugely important! Mass spec is the most important tool in our arsenal thanks to its high sensitivity and ability to handle complex mixtures. For me, it's the most important tool in environmental research because we can use it to identify new contaminants, quantify contaminants, and so on. If NMR was this sensitive and could handle mixtures, maybe we'd veer away from mass spec. But as it stands, mass spec remains on top!

Is there anything missing from the analytical toolbox for environmental analysis?

Some people in the field have started using supercritical fluid chromatography (SFC)-MS. Only time will tell if this fills an important niche. One thing we're missing is an automatic process for testing toxicity of collected prep-LC-MS fractions. As other areas of analytical chemistry begin to use AI, maybe environmental research should take inspiration to push EDA into the future.

Any advice for the next generation of analytical scientists working in environmental analysis?

There's so much to discover and do – newly emerging analytical scientists have a wonderful opportunity to make a difference in human and environmental health. Alongside grasping every opportunity that presents itself, it's crucial that you don't give up! There's always pitfalls, but with collaboration and determination, you will achieve exciting results.

What are your hopes for the future?

I'm hopeful that as the field evolves, we will identify important new contaminants that allow us to draft solutions to minimize human and ecological exposure.

References

1. *Z Tian et al., Science, 371, 6525 (2020). DOI: 10.1126/science.abd6951.*
2. *S Breinlinger et al., Science, 371, 6536 (2021). DOI: 10.1126/science.aax9050.*
3. *JM Allen et al., Environ Sci Technol, 56, 1 (2022). DOI: 10.1021/acs.est.1c07998.*
4. *J Li et al., Environ Sci Technol, 56, 16 (2022). DOI: 10.1021/acs.est.2c02490.*
5. *H Dong et al., Environ Sci Technol, 57, 47 (2023). DOI: 10.1021/acs.est.3c00771.*

ORIGIN STORY

At graduate school, I was not focused on environmental research. I was not even studying analytical chemistry; I was doing physical/organic chemistry. But I had the opportunity to run the mass spectrometry lab in the chemistry department and gained some good experience using MS, which led me into learning how to identify unknowns in samples.

When I was close to graduating, the general chem labs professor at Emory University told me that I should apply to the EPA National Exposure lab in Athens, Georgia. There wasn't an opening at the time, I just sent a cold resume, but it turned out they had purchased the same really advanced HR magnetic sector mass spectrometer that I had been operating – and were clueless on how to use it!

I didn't know anything about environmental chemistry until I joined EPA. It was all on-the-job training. I had experience identifying unknowns using HRMS, but now I had to apply it to environmental problems. I went to scientific conferences, I read the literature and learnt as I went – and I loved it. I morphed into this role.

About two years into my time at EPA, two scientists came to us – one from EPA Cincinnati and one from the University of North Carolina, Chapel Hill. They were both doing research on DBPs and, knowing that we had expertise in identifying unknown chemicals in water, they asked us to collaborate with them on a couple of DBP projects. At that time, there were very few people looking at drinking water; almost everybody was doing only the regulated DBPs. I recognized that it was an important problem, and we decided to combine their extensive expertise in DBPs with my background in identifying unknowns.

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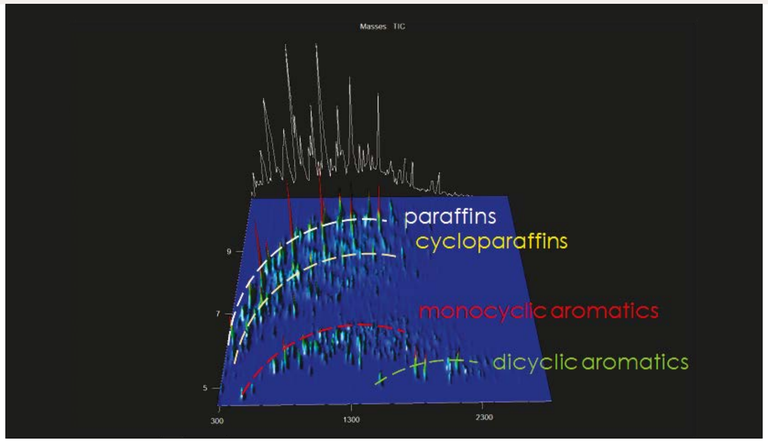
APPLICATION NOTE

Characterization of Fischer-Tropsch Synthetic Paraffinic Kerosene and Traditional Aviation Turbine Fuel

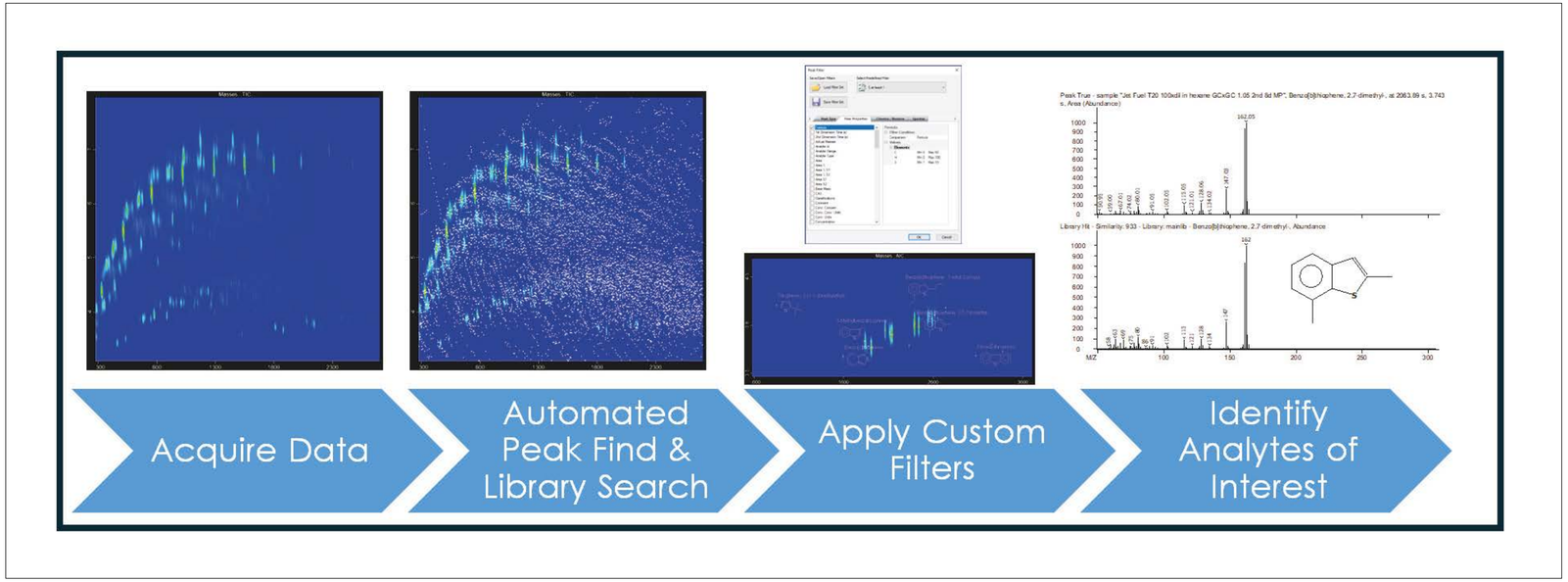
The Synthetic Aviation Fuels (SAF) market offers a sustainable alternative to fossil-based aviation fuels

The synthetic aviation fuels (SAF) market has seen recent growth that is expected to continue thanks to regulations like the European Union's "ReFuel EU" proposal and the United States Sustainable Aviation Fuel Grand Challenge. SAFs derived from sustainable and renewable resources are less impactful on-air quality than traditional fossil-based aviation fuels due to their processing pathways reducing the total aromatics and sulfur content. However, a certain amount of aromatic content is still necessary to maintain the proper freeze points, viscosity, and polymeric sealing properties in a jet fueling system. Thus, it is important to know the physical properties of a new fuel and the chemical makeup. With a combination of comprehensive two-dimensional gas chromatography (GCxGC) and time-of-flight mass spectrometry (TOFMS), the high-quality information necessary for a deeper understanding of the composition of synthetic aviation fuels can be produced and utilized to expedite the certification process and gain approval for use.

LEARN MORE WITH OUR SAF APP NOTE



GCxGC 3D surface plot of commercial aviation turbine fuel with Total Ion Chromatogram (TIC) shown. Reconstructed trace of what a single dimension of GC separation would have looked like is shown in white. Elution bands of paraffins (white), cycloparaffins (yellow), monocyclic aromatics (red), and dicyclic aromatics (green) are indicated by dotted lines.



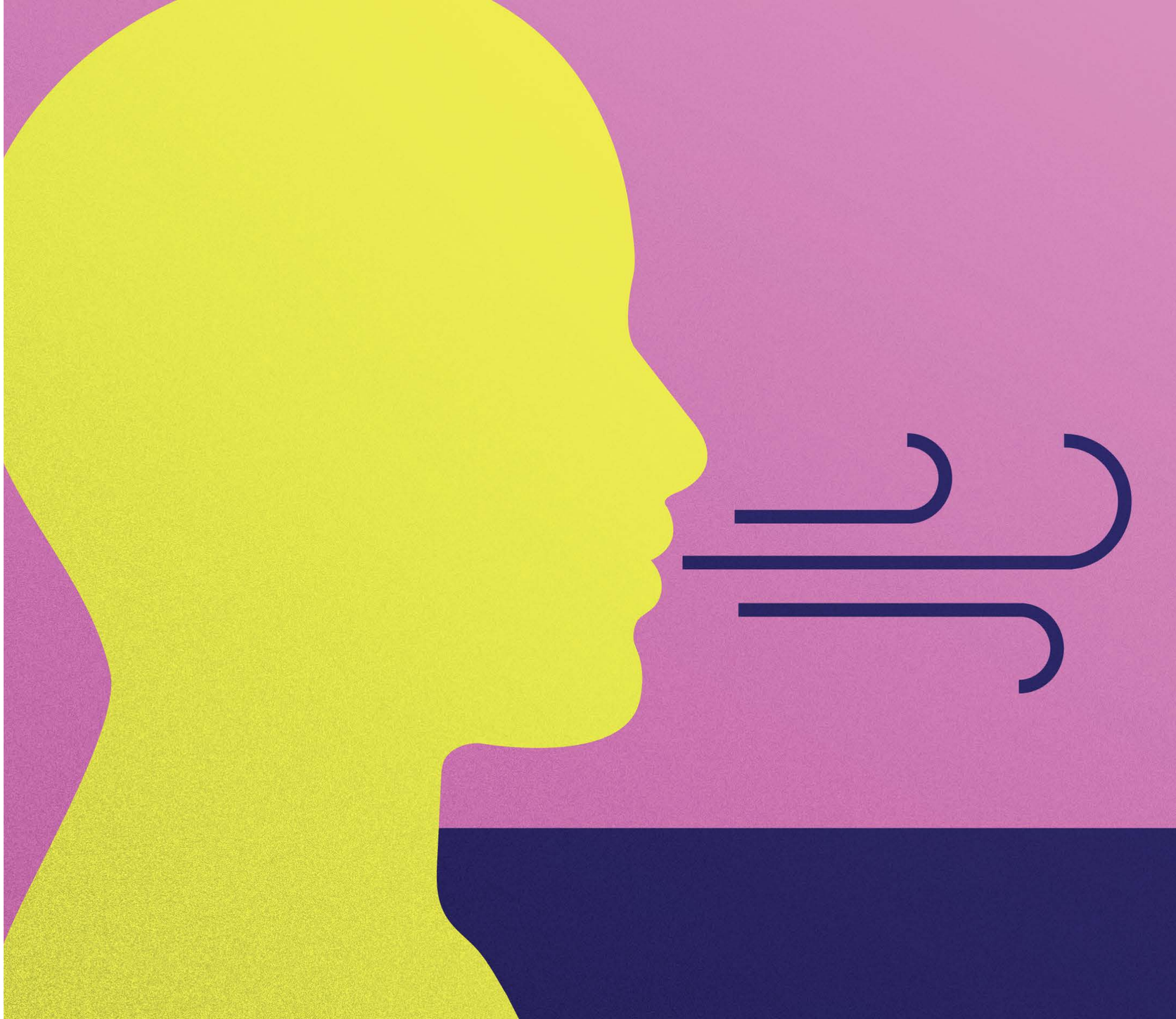
Data processing workflow for semi-targeted analysis of traditional aviation fuel, showing the power of custom peak filtering for narrowing focus to potential analytes of interest.

FEATURE

The Exhaled Microbiome Profile

How volatile compounds in breath can be used to diagnose disease, develop personalized therapies, and combat antibiotic resistance

By Tina Chou



In recent years, breath analysis has emerged as a non-invasive and promising tool for disease diagnosis. Hundreds of chemical compounds are encompassed within a breath sample, and volatile organic compounds (VOCs) in particular can be derived from metabolic processes. Measuring VOCs in breath samples provides a snapshot of the physiological status of bodily functions – the composition and abundance reflecting metabolic changes that could be indicative of disease.

Unlike current sampling methodologies, which mainly focus on fecal matter and blood, breath collection provides a non-invasive and convenient method to real-time testing. As a waste product constantly emitted from the body, breath sampling enables researchers to take repeated measurements over a short timeframe with minimal discomfort to the patient.

In 2020, The Analytical Scientist published a feature on making the "breath biopsy" a reality. This article will focus on recent advances in our understanding of how microbiome-produced VOCs in breath add to the picture.

Breath analysis of the gut microbiome

Many studies have demonstrated distinct breath VOC profiles amongst individuals with and without specific diseases. Such VOCs

have the potential to distinguish individuals with particular diseases, facilitate diagnosis, and provide a tool for monitoring disease progression and treatment efficacy.

Various analytical approaches can identify VOCs in a breath sample. However, gas chromatography-mass spectrometry (GC-MS) is widely accepted as the gold standard due to its high sensitivity and accuracy. Depending on the goals of the study, additional computational approaches – such as regression analysis – can generate discriminatory tests from breath samples. After identifying molecular features via a mass spectrum in GC-MS analysis, these features are matched to libraries like the National Institute of Standards and Technology (NIST) for tentative identifications.

VOCs produced by gut microbes – such as short-chain fatty acids (SCFA), indole, trimethylamine, and other compounds involved in different metabolic pathways – can also be found in breath samples. The changes in abundance of these compounds in traditional sampling matrices (fecal matter and blood) suggest the involvement of the gut microbiome in gastrointestinal diseases and cardiovascular metabolic diseases (1–4).

The gut microbiome in itself is highly diverse and variable depending on several factors, including lifestyle, age, and overall health. Differences in the community of microbes have also been associated with the development of inflammatory bowel disease (IBD) (5, 6) and cancer (7, 8).

A study in 2018 demonstrated the impact of the microbiome in Crohn's disease – a subtype of IBD (9). During active and remission status, individuals provided breath samples for non-targeted VOC analysis and fecal samples for metagenomics analysis. The former approach doesn't require prior VOC identification, allowing for the discovery of novel biomarkers. Additionally, this approach captures all the compounds reflecting overall physiological changes, decreasing bias, and improving ideology for exploratory studies.

In this particular study, compounds were analyzed with thermal-desorption GC-MS, with wavelets and P-splines applied to reduce background noise and correct baseline respectively. To determine which compounds were the same, the area under each peak of the total ion current (TIC) chromatograms and similarities in retention time were calculated, while the correlation of mass spectra were matched among similar samples. A data matrix with patients and relative concentrations of VOCs were also created for correction analysis.

Though the authors did not validate identified compounds with a chemical standard, the results showed that, regardless of disease status, the tentatively identified SCFAs, acetate, and propionate were significantly correlated with levels of Bifidobacteria and several other microbes in the Firmicutes phylum – all of which were implicated in the development of Crohn's disease.

Additionally, the abundance of these VOCs and their correlated microbial strains decreased in subjects during their disease state relative to their remission state. This demonstrates how microbial-produced breath VOCs, the gut microbiota, and disease state are implicitly connected.

Gut microbial activity in drug development

With further research into microbiome-produced VOCs in breath, we could see a revolution in disease diagnosis and monitoring for liver disease.

Non-alcoholic fatty acid liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) are thought to share common physiological causes with alcoholic fatty liver disease (AFLD). Recent studies concluded that microbial dysbiosis may be associated with the development of NAFLD and NASH through overproduction of ethanol by certain bacteria, such as Klebsiella pneumoniae (10, 11). In other words, without consuming alcoholic beverages, the bacterial-derived ethanol is high enough to exceed the recommended daily amount.

These findings could have a knock-on effect on the pharmaceutical industry, given that nearly half of prescribed medications have potential interactions with alcohol. As shown in Figure 1 below, there are at least eight classes of drugs in development for NASH treatment – each acting against different relevant targets to alleviate symptoms or slow disease progression. Disregarding gut ethanol production during the development of these treatments could lead to clinical trial failure and reduce the chances of establishing drug efficacy. A diagnostic test for bacterial ethanol production could also help identify individuals at risk of high ethanol exposure prior to clinical visits, enabling better selection of patients for drug trials or treatment.

As ethanol is metabolized by alcohol dehydrogenase in the liver before reaching the peripheral circulation, concentrations of bacterial-derived ethanol in exhaled breath are expected to be less than 20 ppb (parts per billion) – much lower than the current limit of detection of commercially available breathalyzers. Lab-based analytical instruments are capable of detecting breath ethanol in the ppq (parts per quadrillion) range and, with further development, can be translated to handheld sampling devices for at-home testing.

Fighting antimicrobial resistance

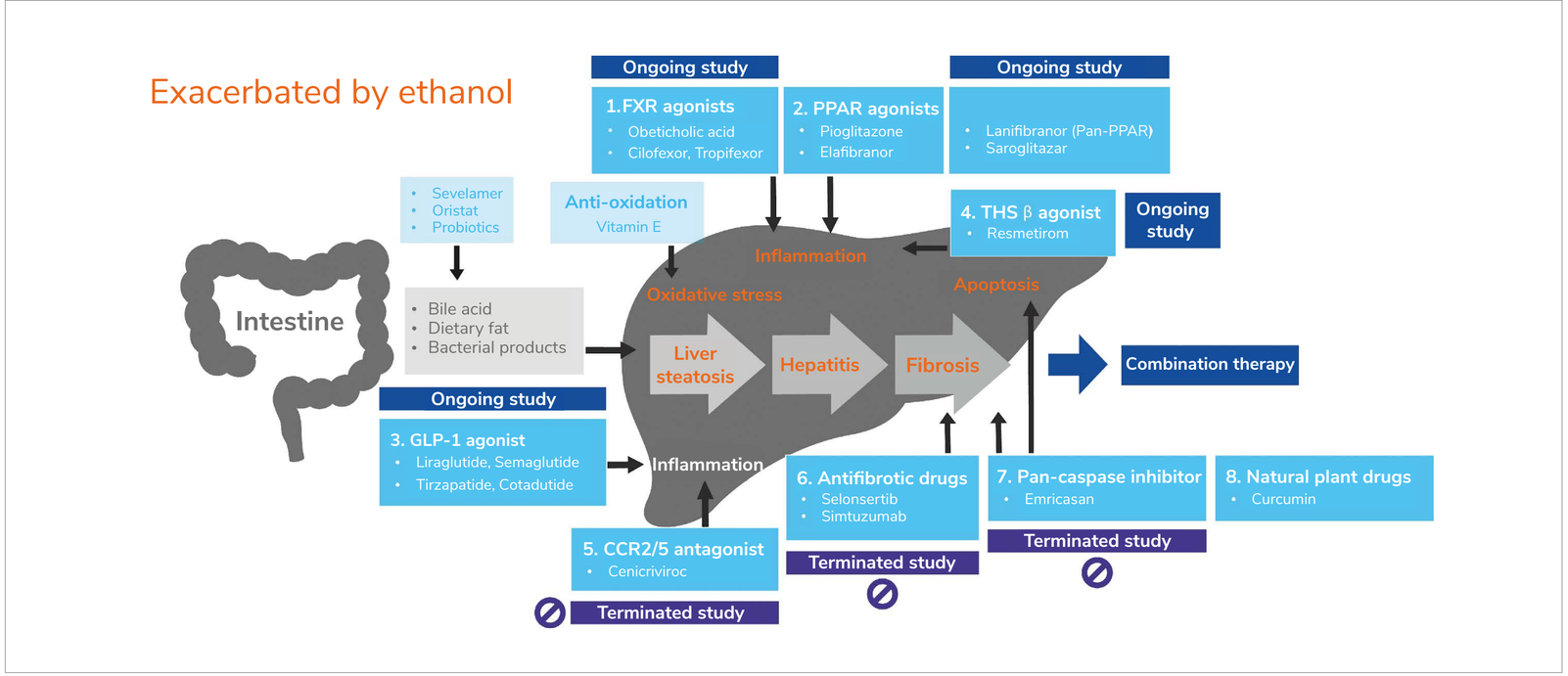
The intersection between breath analysis and the human microbiome does not stop at disease diagnostics and treatment monitoring. Integration of breath and microbiome research can provide further insight into rising antimicrobial resistance concerns – potentially improving clinicians' ability to reduce this issue alongside other health risks through tailored therapy.

A pilot study hypothesized that the treatment pathway and chosen antibiotic may have varying effects on the patient's gut microbiome – with changes reflected in breath volatile metabolites (12). Over a 12 month period, the patient's gut microbiome and breath volatile metabolite data were recorded before, during, and after antibiotic therapy. Breath samples were analyzed through GC-MS, while microbial DNA from stools were analyzed through long and short-read DNA sequencing.

The relationship between breath and gut microbiota in this study was less clear for several reasons, including a lack of full representation of the gut microbiome in fecal matter. Additionally, the study conducted a targeted analysis, which didn't represent the whole metabolome and could have missed crucial information.

However, an interesting result was found in one of the patients referred to prolonged antimicrobial therapy due to an unusual clinical history, with 11 years of biliary system infection. This patient had consistently high concentrations of all methyl halides, which negatively correlated with three specific gut bacteria. Between pre and post-therapy, a shift in the dominant Bacteroides strain was noted in this patient, along with altered breath metabolite composition.

Though the patient's health did improve over time, most breath VOCs didn't return to their baseline values, suggesting that microbial or human metabolism was substantially modified during recovery and/or antimicrobial treatment. The longer-term effects of microbial changes and the VOCs produced through treatment remain unknown



– further research into these specific details is required. However, the case report shows the significance of comparing longitudinal pattern changes: understanding the effects of antimicrobial exposure through breath and microbiomes is an important factor in improving personalized medication in the years to come.

The need for breath sampling

Microbial-produced VOCs are typically more abundant than those endogenously produced in a breath sample, but volatile compounds in general are in low abundance in exhaled breath. Therefore, there is a crucial challenge in effectively reducing noise in samples and enhancing the sensitivity of compound detection. Apart from restricting test subjects' behaviors (such as drinking, eating, and smoking) prior to breath sampling, noise reduction can be achieved by ensuring the compound measured is genuinely "on-breath."

Once identified and validated, breath biomarkers can be used for a targeted chemical analysis approach, with less concern for background reduction. A validated biomarker or a panel of biomarkers can be leveraged by technology like field asymmetric ion mobility spectrometry (FAIMS) or metal oxide sensors to generate a handheld device for clinical tests. For example, hydrogen-methane sensor-based tests provide rapid, accurate, and cost-effective diagnosis for gastrointestinal conditions, such as small intestinal bacterial overgrowth (SIBO) and carbohydrate malabsorption.

Overall, breath sampling provides researchers a non-invasive and user-friendly approach to obtain real-time data reflecting changes in microbial-produced metabolites. This enables informed decision-making in drug development and the customization of treatment plans. With a vast range of application possibilities, there is a clear demand for an effective breath sampling device to analyze the metabolic activity of the gut microbiome and the screening and diagnosis of associated diseases.

Tina Chou is Senior Biomarker Scientist, Owlstone Medical, Cambridge, UK

REFERENCES AVAILABLE ONLINE

FEATURE

The GC-MS Application Challenge

We catch up with 2023's GC-MS Application Challenge winners

The Analytical Scientist, in collaboration with LECO Corporation, Restek, Axel Semrau and GL Sciences, invited those working in the GC-MS space to submit their most impressive application notes for the chance to win some exciting prizes – all expenses paid facility or conference trips, consumables worth $3,000, and instrument discounts, to name but a few.

Our expert panel of judges – including Robert K. Nelson, James Harynuk, Susan Richardson, Hans-Gerd Janssen, Giorgia Purcaro, Erich Leitner, Jaap de Zeeuw, and Robert Trengove – have considered the entries and the results are in! Our winners across the following categories are:

- Dmitry Koluntaev for Best Novel Application
- Anika Lokker for Excellence in Chromatography
- Flavio A. Franchina for Creative Use of Application Workflows, Sample Prep & Automation
- Katelynn Perrault – Special Recognition

We caught up with the winners to find out more about their work, the lessons learned, and thoughts on the future of GC-MS.

Who's Afraid of Picasso?

Best Novel Application: "Analytical approach to GC-MS determination of museum varnish compositions"

With Dmitry Koluntaev, Application Specialist, Q-Tek, Montenegro

Please introduce yourself…

As an application specialist, I work for a small private company that manufactures GC-MS systems. Searching for new applications for GC-MS is one of our main focuses.

I am a biologist by education, but I have been working with mass spectrometry since university – it's become a great hobby! I really enjoy discovering new possibilities for GC-MS; for example, in museum object analysis.

What was your main inspiration?

I always wanted to work with samples filled with history – and around a year ago, we were asked for assistance by restorers to analyze samples of varnish taken from two museum items – book covers and chest lids. It was very important to determine exactly what type of varnish had been originally used by the artist so that it could be properly removed – without damaging the drawings underneath. We agreed! It was a great opportunity to try our hand at such a field of research and use real historical samples for analysis.

I spent several days analyzing the literature to find out what types of varnishes had been used back then, how they had been prepared by masters, and what recipes had been used. Having studied the objects of research, I started looking for various samples of varnish in specialized art stores. Developing a specialized library of markers for each type of varnish became the optimal solution for discovering the steps of sample preparation.

The aspiration to find the answer to the question posed and the desire to touch historical subjects – that's what really fascinated me in this work.

Any challenges?

To prevent harm to the picture, historical research usually involves incredibly small samples. And for this study, all stages of sample preparation had to be efficient and universal – meaning that one extraction method was used across a variety of target compounds; there was no possibility of re-analysis. To uphold these requirements, we implemented a unified method of sample preparation that would allow determination of resin markers and oils in which the artist melted the resin and prepared the varnish.

Another challenge of our research was that the obtained chromatographic profiles of the resin extracts are usually complex chromatograms, which are difficult to interpret without using resins of known botanical origin. Therefore, it was necessary to develop a list of characteristic markers for each type of resin.

Any lessons learned?

Having immersed myself in the literature, I noticed the broad potential of using GC-MS in the study of museum objects. For example, characterization of the binding oils used by artists in painting is in high demand not only in the process of restoration of an object of art, but also in answering the question about the date of painting to confirm its authenticity.

Another main takeaway from this study was that a large extent of any task can be solved and there is nothing to be afraid of when approaching your research. A thorough study of the objects of research at the initial stage

allows you to choose the direction of the study. Working with intermediate results also allows you to assess their correlation with expected results.

Do you have any tips for scientists hoping to bring a touch of creative flair to their application workflows or method development?

By combining the exploration of new GC-MS trends, tracking applications from major manufacturers, and reading scientific reviews, we can find answers to questions that may otherwise have been unsolved. You must go beyond the framework to grow and develop as an analytical scientist – this is where creativity and discovery lies.

What applications do you hope to explore in the future?

I remain deeply immersed in exploring the possibilities that GC-MS offers in targeted and non-targeted metabolomics. Today, we have many opportunities to use open-source software for processing mass spectrometry data (for example, MzMine, GNPS, XCMS, MetaboAnalyst), which allow us to use GC-MS systems in new ways, access harmonized data, and visualize interpretation of results.

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Getting to Grips with Prehistoric Adhesives

Excellence in Chromatography: "Breaking the secret of prehistoric stone tools design using multidimensional chromatography"

With Anika Lokker, PhD student, University of Liège, Belgium

Please introduce yourself…

I am an analytical chemistry PhD student with a heart for archaeological research. From a young age, I was interested in history (archaeology, in particular) and science. Combining both of these areas for my PhD project has been very stimulating.

My PhD project focuses on non-destructive identification of prehistoric adhesives, which could have been used to attach a handle to the tool or as a protective wrapping. Resins, waxes, gums, animal glues, tars, and dry distillation of birch bark might have been used as adhesives in these times. Since the adhesives tend to remain present on stone tools, whereas the organic handle is often gone, the analysis of adhesives is important; it can tell a lot about how the tool was used.

We are currently developing a non-destructive analysis method, using the volatile organic compounds (VOCs) released by the adhesives. VOCs are trapped with dynamic headspace (DHS) before being separated and detected with comprehensive two-dimensional GC-TOFMS (GC×GC-TOFMS). The technique is fully automated with a multipurpose sampler (MPS). The main focus of the project is on artifacts from several excavation sites in South Africa from the Middle to the Late Stone Age (150,000–20,000 years ago).

This project is interdisciplinary, with two groups from different faculties working closely together – the analytical chemistry group of Jef Focant (OBiAChem, Faculty of Sciences, ULiège) and the prehistoric research group of Veerle Rots (Traceolab, Faculty of Human Sciences, ULiège).

Another PhD researcher and a postdoc researcher are working alongside me with a focus on functional analysis of stone tools, which entails technological use-wear and residue analysis. This process involves several non-destructive microscopic and spectroscopic techniques. Together, we are trying to understand the life cycle of stone tools.

How did you become involved with this project?

A few years ago, a proof-of-concept study was conducted in my lab using HS-SPME-GC×GC-TOFMS to identify prehistoric adhesives. I started my PhD project with this technique, too.

However, it soon became clear that HS-SPME is not sensitive enough to detect the VOCs of very old archaeological artifacts. Therefore, I started to look further into different HS techniques options, finding articles in which they compared HS-SPME with DHS. I discovered that DHS has a higher response and sensitivity. With this knowledge, we changed our system to use the potential of DHS and optimized the extraction. This change showcased the measurement of a real archeological artifact and highlighted the working benefits of DHS.

What were the main challenges?

The results were not very promising at the beginning of the study. I was struggling to optimize the method of DHS – it didn't look much better than HS-SPME in terms of sensitivity. It also took some time to familiarize myself with this method as it wasn't used much within our lab. It was only after the discovery of the design of experiments concept that I managed to break through this barrier.

What were your main findings?

Using DHS allowed us to detect VOCs from a very old artifact with more success than HS-SPME. We're only at the beginning of the project, but the results with DHS look very promising in being able to identify the adhesives used on ancient artifacts.

Any lessons learned?

In this line of research, I am constantly learning new things, especially because I just started working with this kind of instrumentation. One thing that I can take forward from this project is to have patience – things will work out eventually if you are determined; troubleshooting (a lot) is part of the job!

Do you have any tips for scientists hoping to bring a touch of creative flair to their application workflows or method development?

It might be obvious, but keep expanding your search for interesting applications – go broader than the intentional field of interest. If you face a problem with the current applications in your research field, try to look for other methods applied to another research field with similar molecules of interest; I took a lot of inspiration from DHS applied to food and beverages research and applied it to archaeology.

What comes next?

I'm currently planning to continue work on my PhD project and dive deeper into what DHS can mean for prehistoric adhesives – we're just getting started! One thing we are planning to do is look into artificial aging of the adhesives. We expect that fresh adhesives will have a different VOCs profile to aged adhesives, but only time and research will tell.

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The Killer GC-MS Application?

Flavio A. Franchina:

The multivariate information received by hyphenating gas chromatography and mass spectrometry make the combination the most suitable technique for broad non-targeted analyses, in my view. For example, the quest for novel energies and materials, where it is important to know, in depth, the composition of the new feedstock in order to finely tune the process. The high-selectivity and sensitivity of the GC-MS also make it also suitable for targeted analysis.

Lastly, when considering the evolution of GC and MS into multidimensional and high-resolution coupled together – i.e., GC×GC-HR MS – then it's possible to combine either (multi)targeted, non-targeted, and post-targeted (retrospective) analyses into a single experimental analytical pipeline, reducing cost and time. Such a high-resolution technique can untangle the complex information contained in biological samples.

However, it's important to doubly underline that such powerful high-resolution couplings aren't magic and require broad understanding and/or training. For example, for the best results, the separation step must also be in harmony with proper sample preparation techniques and experimental designs.

Dmitry Koluntaev:

It's very difficult to imagine. I suppose such an application should be very simple in terms of sample preparation (maybe even fully automated), and should provide a comprehensive analysis of any object – whether it be a museum object or the analysis of biological or environmental objects.

For example, there are interesting studies with exhaled breath taking place in the world. I try to actively follow the publications and reports in this area.

Anika Lokker:

I'm interested in the development of non-destructive GC-MS analysis in cultural heritage research. HS-GC-MS analysis is already gaining more popularity in the field and l am curious what kind of applications might be coming in the future.

Katelynn Perrault:

I am really interested in the application of GC×GC-MS to non-targeted profiling in forensic investigations. There are several areas where GC×GC could be used on a more regular and routine basis. We hope to investigate some of these applications in the future to improve complex chemical separations that can be applied in legal investigations.

Creatively Controlling Pesticides

Creative Use of Application Workflows, Sample Prep & Automation: "Determination of phytosanitary products in surface waters and groundwaters by GC×GC-TOFMS"

With Flavio A. Franchina, Assistant Professor of Analytical Chemistry at the University of Ferrara, Italy

Please introduce yourself…

My research is focused on the implementing and integrating into analytical workflows effective sample preparation, separation, detection, and data elaboration techniques for targeted and non-targeted analysis of small molecules. We apply these strategies and finely tailor them to tackle challenges in food, biomedical, and environmental applications.

Here, we described the development and validation of a method for the determination of phytosanitary compounds in environmental waters. For this, we relied on a solid-phase extraction (according to the EPA method 3535A), followed by injection into a comprehensive two-dimensional gas chromatography coupled to mass spectrometry (GC×GC-MS) system.

What was your main inspiration?

The idea popped into my mind when tutoring one of my students, who was working on his dissertation. At the time, he was working part time for one of the national environmental agency's local labs, so I had the chance to hear about some of the analytical challenges they faced. I thought that developing a GC×GC-MS methodology for phytosanitary compounds could really be helpful, and that comparing it with its GC counterpart would be interesting – and a good learning exercise for the team, both in terms of theoretical and technical skill development.

What were your main findings?

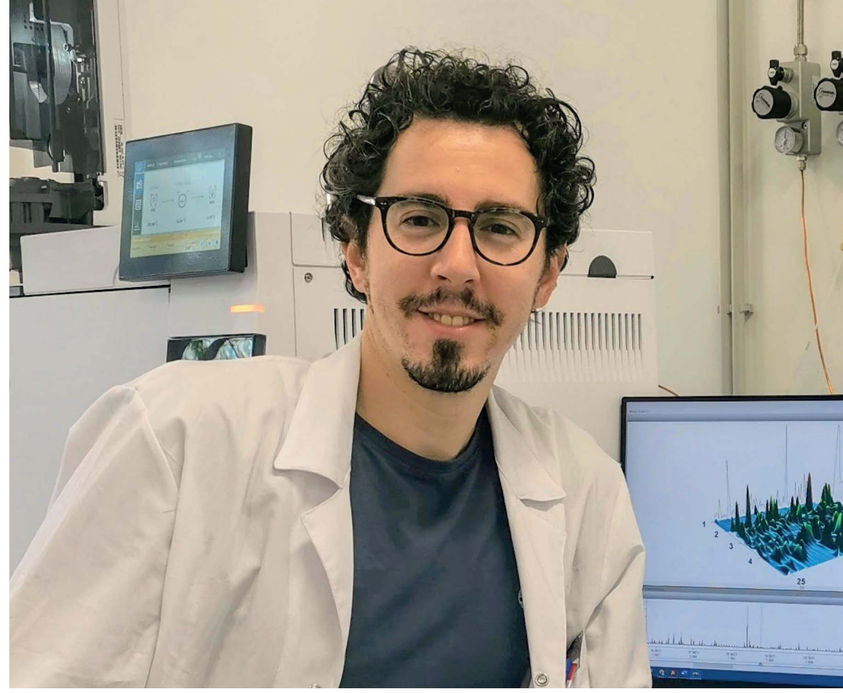
We analyzed some real-world water samples in collaboration with the national environmental agency, and we found that – fortunately – the total amount of phytosanitary compounds was below the legal limit for most of them. The occurrence of contamination was also more evident among superficial water samples, compared to groundwaters. Curiously, the most common compound detected over the limit (0.1 µg/L) was caffeine.

Any challenges?

As shown in some of the figures of the application, 1D GC separation was insufficient to resolve some target peaks, which co-eluted with other species. Depending on the case, some of them were spectrally resolved by the MS, thus a single separation would successfully do the job; others instead greatly benefitted from the additional separation into the second dimension with the GC×GC method. We also quickly realized the need to develop quality control procedures because the extracts and standards were not very stable during storage.

Do you have any tips for scientists hoping to bring a touch of creative flair to their application workflows or method development?

I think it's important to acquire a solid and deep knowledge of specific topics or techniques; then, contaminations from other fundamental disciplines might let you find different angles for the topic you master.



Looking at something that you know well from a different perspective is a way to exercise your creativity and make a difference.

What applications do you hope to explore in the future?

I would like to develop and transfer more informative and effective analytical strategies to companies' R&D and QC laboratories.

Regarding the environmental monitoring, we are more recently using a programmable-temperature vaporizer for the GC×GC-MS system. I believe this can greatly help to reduce solvent consumption during the extraction process.

We're also working on the development of robust methodologies of chemical analysis for the investigation of metabolites in clinical settings... Stay tuned!

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Sandalwood in the Second Dimension

Special Recognition: "Comprehensive two-dimensional gas chromatography analysis of commercial essential oils from different sandalwood species"

With Katelynn A. Perrault, Associate Professor of Forensic Sciences and Chemistry and joined Chaminade University of Honolulu, USA

Please introduce yourself…

Our research involves the non-targeted profiling of complex samples by comprehensive two-dimensional gas chromatography (GC×GC). Specifically, we focus on volatile matrices that comprise complex odors related to forensic science, biomedical, and natural product applications.

What was your main inspiration?

Recently, we worked on several complex samples of Pacific Island origin due to our location in the Pacific and our interest in complex plant products in Hawaii. These included samples of kava (a beverage made from Piper methysticum) and poi (food product from Colocasia esculenta).

Many personal care and aromatherapy products in Hawaii contain Royal Hawaiian Sandalwood within them, and we were curious to see if our non-targeted methods could differentiate the entirety of the volatile profile of other sandalwood species from other regions in the world. This was particularly interesting because some ISO methods are developed to look at quality indicators within sandalwood essential oils, but they are based on targeted analysis of only a few compounds. Our non-targeted GC×GC method allows us to see a wealth of information within these complex plant products. With this knowledge, we were interested to see if the method would provide further value in differentiating the oils.

Any challenges?

We had to optimize our method to improve our separation because the samples were quite different from other samples we analyze. The samples were injected using our one-dimensional GC instrument to see what the samples contained. I tasked my Instrumental Analysis class (in groups) to come up with different ways of improving the 1D GC separation by playing with parameters, such as oven ramp, split flow, and carrier gas flow rate.

Taking the optimized 1D GC method, we translated it to our GC×GC system for further analysis. The students had a lot of fun learning about the GC technique using this "optimization challenge" and it brought up some great points of discussion of chromatographic theory in class after the results were obtained.

What were your main findings?

GC×GC was a powerful tool to elucidate subtle but important changes in the complex chemical sandalwood essential oil profiles. Non-targeted analysis was used to investigate compounds that are quality indicators – according to standard methods – simultaneously with other product components that could be relevant to monitoring these products. Monitoring additional compounds within the profile may assist in understanding their different therapeutic benefits by tracking a component to its known pharmacology.

Any lessons learned?

During the study, we were contacted by representatives of an essential oil company who asked how GC×GC might help them with product monitoring. Having data to share with them and explain the benefits of GC×GC was incredibly valuable.

Do you have any tips for scientists hoping to bring a touch of creative flair to their application workflows or method development?

Crowdsourcing ideas for method development can be valuable. For our study, a group of undergraduate students came up with ideas for optimizing our first dimension separation – a fun exercise to tackle together. I could also see this group approach being a valuable tool in R&D laboratories when meeting challenges in method development.

What's on the application horizon?

There is always a long wishlist of new applications that we want to tackle; you will have to wait and see what we do next!

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