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Jennifer M. Nguyen, Waters Corporation
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Innovation in Nutrition and Food Analysis: The FoodOmicsGR Research Infrastructure
Petros Pousinis1,2,3, Alexandros Pechlivanis1,2,3, Artemis Lioupi1,2,3, Helen Gika2,4, Christina Virgiliou1,2,3, Dritan Kodra1,2,3, Maria Marinaki1,2,3, and Georgios Theodoridis1,2,3
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This article describes a new approach for measuring critical quality attributes including the confirmation of 5’ cap structure and 5’ capping efficiency.

Three-Dimensional Printing in Ion Mobility and Mass Spectrometry
Interest in three-dimensional (3D) printing technology is expanding rapidly. What impact can it have in mass spectrometry (MS)? Robert Winkler, of the Center for Research and Advanced Studies Irapuato, in Guanajuato, Mexico, is exploring this question. We recently spoke with Prof. Winkler about this work.

Are you using your pipette properly?
The Power of the Pipette
The latest research news and news in brief

The Analytical Arsenal for Point Source Attribution
Zacariah L. Hildenbrand1 and Kevin A. Schug2, 1The University of Texas at El Paso, 2The University of Texas (UT) at Arlington
How can we monitor the source and environmental impact of neglected oil and gas acquisition sites, and assess their impact over time?
Sweating the Small Stuff—Are You Sure You Are Using Your Pipette Properly?

Most analytical chemists believe their pipetting technique is infallible, but few of us are actually following all of the recommendations within the relevant guidance. Incognito investigates good pipetting practice and busts some of the urban myths behind what is probably the most widely used analytical tool.

At the heart of most analytical techniques is sample preparation. At the heart of most sample preparation is dilution and quantitative transfer of liquids. In many cases, these operations are performed using air displacement or positive displacement pipettes, which I’d like to focus on in this Incognito column.

You may ask why? Given that these pipettes are used daily by most practicing analytical chemists, surely we know everything there is to know? Well, the trouble is I believe many of us think we know all there is to know and that our pipetting technique is infallible. I guess I was in the same camp until recently, when a laboratory investigation caused me to question just how good I was. Had I been taught properly in the first instance? Had I become lazy over the years? Did I rely too much on the urban myths that had been relayed to me over my career about what’s good and what’s not? It turns out the answer to these questions—in my case—was a pretty emphatic “yes”. So where do I turn for a primary reference to improve my practice and bust some of these myths?
After a little searching, I came across what I believe to be two excellent reference documents on pipettes and the art of pipetting: Internal Standard (ISO) 8655-2 Piston-Operated Volumetric Apparatus—Piston Pipettes (1) and (ISO) 8655-6 Piston-Operated Volumetric Apparatus—Gravimetric Methods for the Determination of Measurement Error (2). Much of the “reference” information contained within this article, and the answers to many of my lingering questions, are drawn from these references. I have also used information and guides from several of the leading pipette manufacturers (3,4) and a useful guide from the National Physical Laboratory (UK) (5). These documents also contain a host of useful information and technical reference tables that are not directly mentioned here but which every analytical chemist should be aware of.

For those readers who may be a little sceptical as to how much difference pipetting errors can contribute to the overall uncertainty budget of an analytical measurement, I’ll be highlighting the impact of poor pipetting technique as we progress the discussion.

So, let’s start with choosing the correct pipette for the job. On the basis that I see far more air displacement pipettes in laboratories than positive displacement pipettes, lets look at the working principles of both, and the circumstances under which they should be used.

In short, an air displacement pipette has a layer of air between the head of the piston and the liquid within the pipette tip. Air pressure is used to force liquid from the tip. Positive displacement pipettes have an integral piston within the disposable tip and the piston is in contact with the liquid being dispensed. Both types are shown schematically in Figure 1.

**Figure 1**: Schematic diagram of an air displacement pipette (left) and a positive displacement pipette (right) (image reproduced with permission of CHROMacademy, www.chromacademy.com).

**Figure 2**: Schematic diagram of the various stop positions of a typical manual piston pipette (image reproduced with permission of CHROMacademy, www.chromacademy.com).
• Viscous, volatile, or corrosive samples—positive displacement pipette

I wonder if, like me, you have been using an air displacement pipette for relatively volatile organic solvents for years?

The choice of pipette tips and correct installation also brought further surprises. First, only manufacturers’ tips are recommended, and all manufacturers recommend that if alternative brands are used, then a “test” for accuracy and precision of the dispensed volume should be undertaken, as per ISO 8655-6, which I will cover in more detail later. I was a little sceptical when I read this—is it just a way of locking us into the manufacturer’s consumable? This may be true, but reference 1 does contain a detailed specification for pipette tips and the information that needs to be provided to the user—I’d recommend that anyone purchasing pipette tips reads this.

On pipette tip installation, for air displacement pipettes, rather than pressing the pipette shaft firmly down on to the tip (known as “hammering”) or pressing the tip straight onto the shaft, it is recommended that a slight twisting motion is used to create a good seal. Whilst loose tips may be purchased, it is recommended the tip is always fitted with the piston held vertically to ensure a good seal and so the tip is level on the pipette shaft, and it strikes me that tips supplied in racks are a more convenient (although more costly) option. For positive displacement pipettes, the above guidance should be followed, but once the tip is installed onto the shaft the tip should be lifted out of the rack and the piston lowered until a “click” is heard as the piston locks into the pipette body and the plunger should then be pressed to the first stop position. Figure 2 contains an explanation of the various plunger positions of a typical piston pipette.

Most manufacturers assert the importance of not cleaning and re-using pipette tips.

So now we have chosen the correct pipette type and correctly installed the tip, lets look systematically at the guidance for correct usage; note that I’ve added some notes and interpretation where this brings further clarity. I’ll deal separately with air displacement and positive displacements.

Air Displacement Pipette—Forward Pipetting Mode

a) Set Correct Volume (Variable Volume Pipettes): When using a variable volume pipette, some manufacturers recommend the volume is “dialled” using a clockwise motion. That is, when reducing the volume, slowly move the volume setting dial to the required volume, but when increasing the volume, overshoot the required setting by 1/3 of a turn and then reduce the volume to the desired setting. In this way, the desired volume is always reached using a clockwise motion. Always hold the volume setting at eye level to avoid parallax error.

b) Aspirate an Aliquot of the Liquid to be Dispensed, Allow to Equilibrate, and Dispense to Waste: Holding the pipette vertically, smoothly depress the plunger to the first stop position, immerse the tip of the pipette body into the liquid to be aspirated to the depth recommended in Table 1, and aspirate the liquid by smoothly and steadily raising the plunger to the rest position. Notes: Jerky

<table>
<thead>
<tr>
<th>Pipette Volume (µL)</th>
<th>Immersion Depth (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1–100</td>
<td>2–3</td>
</tr>
<tr>
<td>100–1000</td>
<td>2–4</td>
</tr>
<tr>
<td>1000–5000</td>
<td>2–5</td>
</tr>
</tbody>
</table>

Notes: Jerky
or rapid plunger movement will increase the error in the amounts of liquid aspirated or dispensed. If the tip is immersed too deeply, the increased hydrostatic pressure may cause too much liquid to be aspirated or liquid droplets to adhere to the outside of the pipette tip, which may be transferred to the receiving vessel. If the tip is not immersed deeply enough, then vortexing may occur or the liquid level may fall below the tip before reaching the top of the filling stroke—in both cases too little liquid will be aspirated.

Wait two seconds for the aspirated liquid to move up into the tip and to equilibrate the temperature and pressure of the headspace air above the liquid. Notes: This step is particularly important in air displacement pipettes due to the changing compressibility of the headspace air above the aspirated liquid as a result of temperature and vapour pressure changes. It is also worthy of note that the aspiration of the liquid into the vacuum space created when the plunger is released from the first stop to the rest position is governed by the ambient atmospheric pressure, which may need to be compensated for if significantly different from those at which the pipette is calibrated (typically 20°C, 50% relative humidity, and barometric pressure of 101 kPa).

This first aliquot should then be discarded to waste, or if the liquid is to be retained, return to the original container. Note that any contaminants present on the inside surface of the pipette tip may also be returned to the sample or reagent.

This first aliquot step can significantly reduce the error associated with pipetting and acts to reduce any interfacial effects between the liquid being aspirated and the inside surface of the pipette tip. With certain liquid types, or during pipette calibration or testing, some manufacturers and standards bodies call for up to five pre-equilibration and wetting step repeats prior to the first “real” aspiration into a new tip or when volume changes are made to adjustable pipettes. This may also be necessary in the normal course of operation when dealing with low viscosity or more volatile liquids, especially where a liquid droplet can be seen hanging from the exit orifice of the pipette tip post aspiration.

c) Repeat Step b) but Retain the Aspirate Liquid in the Pipette Tip: Notes: I have read...
The contact between the pipette tip and the wall of the receiving vessel is important to ensure that the liquid is dispensed correctly and to overcome any surface tension effects. One should also note that the ISO guidance recommends a contact angle of between 30 and 45°. It is also important to note that the position of the tip should be above the level of any liquid already within the receiving vessel. If the tip is submerged, then highly inaccurate and poor reproducible volumes will result. It may be necessary to handle the receiving vessel to create the correct contact angle with the pipette; in this case handling should be kept to a minimum (especially when the vessel is to be weighed) and lint-free gloves should be worn.

Smoothly depress the plunger to the first stop position. Wait one second and then smoothly depress the plunger to the second stop position to purge any remaining liquid from the pipette tip.

d) Dispense the Sample: Place the pipette tip gently against the wall of the receiving vessel, just above any liquid that may already be within the vessel, at an angle of between 10 and 45°. Note that this should be achieved by tilting the receiving vessel whilst the pipette remains in the upright orientation.

Notes: It is important that the pipette remains in the upright position during dispensing for the reasons outlined previously, as well as to avoid any contamination of the pipette body by aspirated liquid within the tip running up into the pipette body when tilted. The angle of contact between the pipette tip and the wall of the receiving vessel is important to ensure that the liquid is dispensed correctly and to overcome any surface tension effects. One should also note that the ISO guidance recommends a contact angle of between 30 and 45°. It is also important to note that the position of the tip should be above the level of any liquid already within the receiving vessel. If the tip is submerged, then highly inaccurate and poor reproducible volumes will result. It may be necessary to handle the receiving vessel to create the correct contact angle with the pipette; in this case handling should be kept to a minimum (especially when the vessel is to be weighed) and lint-free gloves should be worn.

Smoothly depress the plunger to the first stop position. Wait one second and then smoothly depress the plunger to the second stop position to purge any remaining liquid from the pipette tip. Notes: In my opinion, this step is one of the most contentious and misunderstood areas of the pipetting technique. Both the guidance and the pipette manufacturers’ literature recommend the use of the “blow-out” or “purge” feature to dispense all liquid from within the tip, although the ISO guidance does state that this should be done “where applicable”. My question is: When is this step applicable and when is it not? I feel it is best practice to use 

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undertake their own experiments to decide if this is necessary to achieve the required accuracy when using the reverse pipetting mode.

Air Displacement Pipette—Reverse Pipetting Mode

Reversed pipetting is particularly useful when handling liquids with high vapour pressure (volatile liquids) or those that are highly viscous. This mode of operation follows all of the guidance that has been discussed above, with some notable exceptions.

a) Prior to aspiration the plunger is depressed to the second stop (purge) position.

b) The liquid is aspirated by allowing the plunger to move from the second stop to the rest position.

c) The liquid is dispensed by pressing the plunger to the first stop position prior to removal.

Notes: The “purge” operation is effectively carried out in the aspiration step rather than the dispensing step. During aspiration, an amount of liquid equal to the amount of air used to purge the tip is aspirated, which compensates for the amount of liquid that remains in the tip after the dispensing step. I have seen no guidance on whether the tip should be drawn along the inner wall prior to removal in the reversed pipetting mode. I would encourage the reader to undertake their own experiments to decide if this is necessary to achieve the required accuracy when using the reverse pipetting mode.

Positive Displacement Pipette

Much of the important guidance has been discussed above, and points around tip wetting, immersion depth, and wiping are applicable. However, once again, there are some notable exceptions.

a) There is only one stop position on these pipettes, any second stop is actually an eject position for the tip.

b) In the dispense step, the piston is moved to the first stop position. The tip is not touched against the side wall of the receiving vessel and is not drawn along the vessel wall prior to removal.

c) There is no reverse pipetting mode.

Having established best practice, it is interesting to discuss sources of error and tolerance of the pipetting equipment, as again there is much debate and, frankly, nonsense spoken about the accuracy and reproducibility of these volumetric tools.

ISO 8655-2 has a useful table of permissible errors for a variety of pipette types, and I’ve shown typical examples from a range of pipette volumes in Table 2.

These figures are applicable for a series of 10-fold measurements where systematic error along the vessel wall, is perhaps an area that is not properly understood. This drawing action is intended to remove any remaining liquid at or around the tip orifice to avoid it being withdrawn by the pipette. Again, some experimentation I have undertaken will be shown later to highlight the effect of not drawing the tip along the pipette wall. It is also important to note that the plunger should not be allowed to move from the second position prior to the contact with the vessel wall being broken (that is, once the tip is removed from the vessel).

<table>
<thead>
<tr>
<th>Error</th>
<th>Effect (Up to %)</th>
<th>Maximum Permissible Random Error (± μL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Badly seated tip</td>
<td>0.5–50.0</td>
<td>Seat tips correctly</td>
</tr>
<tr>
<td>Failure to wipe tip on vessel wall post dispense</td>
<td>3</td>
<td>Ensure air displacement pipette tips are drawn 8–10 mm along the wall of the receiving vessel prior to removal</td>
</tr>
<tr>
<td>Difference in density of liquid being pipetted versus water used to adjust/ calibrate</td>
<td>2</td>
<td>Adjust for density differences</td>
</tr>
<tr>
<td>Pipette tip immersion depth and handling angle</td>
<td>1</td>
<td>Use correct immersion depths and recommended handling angles</td>
</tr>
<tr>
<td>Non-consistent or rapid plunger movement</td>
<td>1.5</td>
<td>Ensure smooth consistent plunger operation</td>
</tr>
<tr>
<td>Uneven piston movement</td>
<td>0.5</td>
<td>Ensure smooth consistent plunger operation</td>
</tr>
<tr>
<td>Straightness of pipette tips</td>
<td>5.0</td>
<td>Do not use pipette tips that are visually deformed</td>
</tr>
<tr>
<td>Failure to pre-wet pipette tip</td>
<td>2.0</td>
<td>Pre-wet tips to ensure volumetric accuracy</td>
</tr>
<tr>
<td>Re-use of pipette tip</td>
<td>4.0</td>
<td>Do not re-use pipette tips unless serially pipetting the same liquid</td>
</tr>
<tr>
<td>Variation between pipette/tip and liquid temperature</td>
<td>0.3%/K</td>
<td>Allow proper thermal equilibration of pipette and liquids</td>
</tr>
</tbody>
</table>

The “purge” operation is effectively carried out in the aspiration step rather than the dispensing step. During aspiration, an amount of liquid equal to the amount of air used to purge the tip is aspirated, which compensates for the amount of liquid that remains in the tip after the dispensing step.

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is the statistical deviation and random error is the coefficient of variation.

It should be noted that whilst the ISO guidance limits for each nominal volume is applicable to every selectable volume throughout the useful volume range of a variable volume pipette, manufacturers often set tighter specification limits for the nominal volume and between the nominal and lower selected volumes.

We have yet to discuss the required adjustments for non-standard environmental conditions or the buoyancy of the liquid used for these measurements, however, I thought it would be interesting to see how accurate my new-found pipetting technique was, using deionized water that meets the criteria of ISO 3696. The results and analysis are shown in Table 3 for an adjustable air displacement pipette of nominal 100 µL volume. It would appear that my technique is reasonable, and I have achieved a reasonable performance against the ISO requirements for each volume:

- Incognito systematic error: +0.57 µL (100) / +0.36 µL (50) / +0.35 µL (10), ISO systematic error limit ± 1.5 μL (mass to volume Z correction factor insignificant);
- Incognito random error: +0.34 µL (100) / +0.11 µL (50) / +0.43 µL (10), ISO systematic error limit ± 0.6 µL (mass to volume Z correction factor insignificant).

It is worthy of note that the error associated with dispensed volume at 10 µL would not have met the ISO tolerances had I used

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**Table 5: Incognito’s “poor practice” errors when using a 100 mL variable volume air displacement pipette**

<table>
<thead>
<tr>
<th>Incorrect Technique Aspect</th>
<th>Systematic Error (µL)</th>
<th>Random Error (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tip immersion depth (1.5 cm)</td>
<td>1.24</td>
<td>0.32</td>
</tr>
<tr>
<td>Non-prewetted tip (different tip for each replicate measurement)</td>
<td>-1.3</td>
<td>0.99</td>
</tr>
<tr>
<td>Failure to wipe tip on vessel wall</td>
<td>-1.1</td>
<td>0.42</td>
</tr>
<tr>
<td>Pipette not kept upright during aspiration and dispense</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>Rapid/inconsistent plunger movement</td>
<td>-2.6</td>
<td>1.7</td>
</tr>
<tr>
<td>Reference ISO limit</td>
<td>+/- 1.5</td>
<td>+/- 0.6</td>
</tr>
<tr>
<td>Error when best practice used</td>
<td>+0.36</td>
<td>+0.11</td>
</tr>
</tbody>
</table>
a pipette of nominal volume 10 µL. This perhaps suggests that one needs to be reasonable about the fraction of the nominal volume that one dispenses, and I know several laboratories who specify that piston pipettes should not be used to dispense volumes less than 50% of the nominal volume for precisely these reasons.

I was also interested in finding out the magnitude of error associated with various aspects of incorrect pipetting technique. The ISO guidance suggests a wide range of estimated errors, some of which are shown in Table 4.

From Table 4 it is obvious that the total cumulative error associated with incorrect pipette technique can be very significant. I investigated the errors associated with some specific aspects of poor technique and these results are shown in Table 5; all results are from 10 replicate measurements of 50 µL of water adjusted for the Z correction factor using a nominal 100 µL pipette.

As can be seen from Table 5, several of the poor techniques resulted in out-of-specification performance for the dispensed volume limits and all increased the inaccuracies relative to that obtained when using best practice. This is perhaps more salutary when considering that each error was implemented in isolation and that the cumulative error, if several of these poor techniques were combined, could be very considerable.

The reader will notice that I have made adjustments to the “expected” mass of water for these experiments in order to account for temperature and pressure differences between my local environment and the standard conditions used to calibrate (adjust) the pipettes (the Z correction factor). This takes me onto my final topic in this discussion—that of pipette testing and calibration, which I’m often asked about, typically by end users who wish to carry out verification between annual calibration and maintenance.

There are two procedures that are defined in ISO 8655-6:2002: the test procedure for more frequent “checking” of the accuracy and reproducibility of the dispensed volume and a calibration procedure, which is typically carried out annually by the pipette manufacturer. This second procedure can also be carried out by the end user with a little experience and some basic metrology tools. Whilst the full details of the test and calibration procedures are outside the scope of this article, I’d like to note several points that are poorly understood:

- Assessment of systematic and random error should be made on 10 replicate measurements.
- Prior to measurement, a tip should be wetted five times and then discarded to equilibrate the air within the pipette to temperature and relative humidity.
- A new tip should be installed for each of the 10 measurements and each tip should be pre-wetted once before aspirating the required volume.
- For measurements below 50 µL, an assessment of evaporation of the test liquid (water to ISO 3696) should be made by measuring mass loss over the same time period as the 10 test measurements and then dividing this mass loss by 10 and adding the resulting mass to each test weighing.
- A correction for temperature and pressure (the Z correction factor) should be made for the mass of water to account for local conditions versus those for adjustment under standard conditions (tables of water mass at various temperature and pressure values can be found in ISO 8655-6).
- For variable volume pipettes, the assessment should be made at the nominal volume, as well as 50% and 10% of the nominal volume (and all must conform to the ISO 8655-6 limits required for the nominal volume).

Note here that the manufacturer may have separate tolerances for the nominal, 50%, and 10% of nominal volumes.

- Systematic error is defined by the variance of the 10 replicate measurements and random error by the coefficient of variation.

I hope that this article acts to stimulate pipette users to examine their own routine and to guide you in best practice as defined by an international standards organization, as well as the pipette manufacturers. I have certainly picked up many tips during the writing of this article and I feel much more confident in my own ability to not only pipette more accurately with piston pipette devices but also to sense when something is not right and to troubleshoot more effectively. To quote Henry Ford, “Anyone who stops learning is old, whether at twenty or eighty. Anyone who keeps learning, stays young”. I feel in the flush of my youth after this salutary learning experience!

References
Analyzing Glycerol in Biodiesel Using SPE and HPLC-RI

Researchers have developed a viable alternative to existing gas chromatography (GC) methods for glycerol determination in biodiesel using solid-phase extraction and high-performance liquid chromatography with a refractive index detector (SPE–HPLC-RI) (1). Derived from the oils and fats of plants or animals, biodiesel is one of many alternatives to crude oil fuel sources. Considered a drop-in biofuel as it is compatible with existing diesel engines and distribution infrastructure, it is usually blended with petrodiesel, with 60 countries around the world mandating its addition. Countries such as the USA, Brazil, China, and Thailand all require petrodiesel to be blended with biodiesel in varying proportions, quite often hovering around the 10% biodiesel to 90% petrodiesel mark (2).

While biodiesel is compatible with modern diesel engines, the sole use of biodiesel as a fuel source is not possible without engine modifications, and in fact biodiesel contains a substance that can cause problems in car engines and in storage infrastructure (1). Glycerol, a co-product of the transesterification of oils and fats, needs to be monitored when blended with traditional petrodiesel. This is because during the blending process high concentrations of glycerol can separate out in the storage tanks. Furthermore, issues such as deposit formation, clogging of fuel injectors, and the production of harmful combustion products, such as aldehydes, are also related to high glycerol concentrations in biodiesel (3).

Currently a technique using GC monitors glycerol concentrations; however, this technique was developed for biodiesels made from canola oil and is not necessarily adequate for biodiesels made from other oils and fats. For example, in Brazil, around 70% of biodiesel is made from soybean oil, with the remaining 30% coming from animal tallow. Analysis with the current standard technique can often yield overlapping peaks, making analysis of biodiesel quality difficult. The great diversity of raw material used for biodiesel production presents many challenges for analytical chemists. Rising to that challenge, researchers aimed to develop a new method to detect and quantify glycerol in biodiesel using SPE and HPLC using a refractive index detector.

The resulting method proved capable of accurately quantifying glycerol in biodiesel samples made from raw materials containing different proportions of soybean oil and tallow. The new method offers a simple, quick, and effective method of determining free glycerol in biodiesel and a viable alternative to existing methods.—L.B.

References
Peaks of the Month

• The LCGC Blog: Full Method Validation is Still a Glaring Deficiency in Many Forensics Laboratories—The deficiency in method validation for forensics laboratories regularly manifests itself in two steps. The first step is where the forensics laboratory does not even provide any indication of method validation when delivering documentation of the case. The second step comes when the documentation made available indicates a lack of rigour in method validation. Read Here>>

• How Much Sample Can I Put on My GC Column?—Here we take an empirical approach and examine concentrations for a wide range of compound polarities on several stationary phases, and determine overload as measured by symmetry. Read Here>>

• Remembering Harold McNair: Three Fundamental Areas of Understanding in Gas Chromatography—Professor Harold Monroe McNair, among the original icons of chromatography, passed away on 27 June 2021 at the age of 88. In this tribute to the remarkable man and using his works as a guide, we explore three fundamental areas of understanding in GC, seeing ideas about problems that still challenge gas chromatographers today. Read Here>>

• Ionization Efficiency for Environmentally Relevant Compounds Using Atmospheric Pressure Photoionization Versus Electrospray Ionization—APPI and ESI are assessed for the MS quantification of pharmaceuticals frequently detected in environmental waters, including antibiotics, beta blockers, and selective-serotonin reuptake inhibitors. Read Here>>

• Trouble Brewing: Categorizing Beers Using LC–QTOF-MS—Beer, beloved by ancient civilizations and modern humans alike for its refreshing and crisp taste, or bitter hoppy taste, or dark oat-laden taste, or light golden taste, and therein lies the problem. The chemical differences come in the specific selection of these ingredients, the addition of any extra ingredients such as fruit, and the exact brewing process used. Researchers used an untargeted LC–QTOF-MS method to analyze 32 beers across five different styles. Read Here>>

News In Brief

Tosoh Celebrates 50th Anniversary of Product Line
Tosoh Bioscience (Tokyo, Japan) is celebrating the 50th anniversary of the introduction of the TSKgel U/HPLC column line. In 1971, Tosoh (known then as Toyo Soda Manufacturing) launched its first gel permeation chromatography (GPC) column for organic-soluble polymer analysis, the TSKgel S-type, a polymer-based column. TSKgel S-type columns paved the way for a suite of columns in nearly every mode of liquid chromatography. Size-exclusion chromatography has continued to remain a special focus of the product line. For more information, please visit: www.tosoh.com

Thermo Fisher Scientific SUT Facility
Thermo Fisher Scientific Inc. (Waltham, Massachusetts, USA) will establish a manufacturing facility in Nashville dedicated to the production of single-use technologies (SUT) products. The facility will more than double the company’s SUT manufacturing capacity in support of its biopharma customers. “Over the last few years, more and more biological drugs and vaccines are being manufactured utilizing the single use platform. This has created incredible demand for our SUT products,” said Michel Lagarde, executive VP of Thermo Fisher Scientific. “The site in Nashville will be critical in Thermo Fisher’s global network to continue to support the biopharma industry’s work in developing life-saving biologics and vaccines.” For more information, please visit: www.thermofisher.com
Innovation in Nutrition and Food Analysis:
The FoodOmicsGR Research Infrastructure

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There has been an increased interest in monitoring food quality and safety, and their important role in relation to human life, wellness, and health. Existing regulations dictate how certain technological processes should be performed to assess food safety and quality by monitoring food components and the potential presence of contaminants. Although the “classical” targeted analytical perspective is still the major route, holistic approaches are gaining more momentum in characterizing food through “omics” technologies because this can provide novel unexpected markers of food quality.

Foodomics (1) combines food and nutrition sciences with advanced analytical techniques and bioinformatics to characterize the food composition or food consumers’ biofluids,
and also to address new challenges such as authentication, traceability, improvement of food produce, food quality, and nutritional value. This holistic approach employs a cutting-edge research infrastructure, where big data are produced that generate new knowledge and highlight previously unknown associations of biomolecules with the studied phenotype.

The potential application of foodomics is very wide and includes the following areas: food characterization and differentiation; food traceability and authenticity; adulteration control; identification of origin (geographic or genetic); monitoring of maturation; assessing freshness; linking organoleptic characteristics with metabolite composition; supporting health claims of novel functional (super) foods; understanding mechanisms of toxicity; development of a personalized dietary intervention for patients with special needs, for example, food allergies; and identifying legitimate or non-legitimate interventions.
Foodomics as an omics approach can provide new knowledge and reveal biochemical mechanisms that describe the advantageous or adverse effects of food components (2). Figure 1 provides an illustration of the fields and the various subfields and applications involved in foodomics.

The Project: FoodOmicsGR_RI

FoodOmicsGR_RI is a national research infrastructure that aims to perform and aid omics research in the agri-food sector in the Greek research environment. This field is of high importance for the country and its agriculture because of the unique landscape Greece exhibits that results in a diverse and rich portfolio of local products and foods. Therefore, the central scope of FoodOmicsGR_RI is to support the Greek agri-food sector by generating robust data on the composition and nutritional value of the local produce, thereby increasing a product’s position, market demand, and provide a higher revenue for the producers.

The project comprises eight Greek universities and research centres. Analytical groups and food specialist groups from the universities of Athens, Crete, the Aegean, Ioannina, the Agricultural University of Athens, the International Hellenic University, and the Biomedical Research Center of the Academy of Athens comprise a team of 60 staff scientists and 40 newly recruited researchers from 20 scientific disciplines. BIOMIC_AUTH, a new interdisciplinary laboratory of the Aristotle University Thessaloniki (http://biomic.web.auth.gr/), with great expertise in bioanalytical chemistry, is coordinating the project. More details can be found on the website (http://foodomics.gr).

Cutting-edge research technologies include high performance liquid chromatography (HPLC), mass spectrometry (MS), nuclear magnetic resonance (NMR) spectroscopy, and inductively coupled plasma–mass spectrometry (ICP–MS) instrumentation and software. These are deployed to cover a wide area of applications such as bioanalysis, metabolomics, genomics, proteomics, and bioinformatics. The capital instrumentation of FoodOmicsGR_RI laboratories includes:

- Five quadrupole time-of-flight (QTOF)-MS instruments (two trapped ion mobility mass spectrometry systems [TIMS]-TOF-MS)
- Three matrix-assisted laser desorption–ionization (MALDI)-TOF-MS instruments
- Four ultrahigh-pressure liquid chromatography (UHPLC)–orbital trap high resolution mass spectrometry (HRMS) instruments (one nano-LC)
- More than 10 UHPLC triple quadrupole MS/MS systems
- Three HPLC–MS single quadrupole systems
- Supercritical fluid chromatography (SFC)–UV/MS system
- More than eight gas chromatography (GC)–MS systems (single quadrupole)
- Four GC–MS/MS systems (triple quadrupoles)
- NMR spectroscopy (600 MHz, 500 MHz, and 400 MHz NMR)
- Two ICP-MS, two ICP-optical emission spectrometry (OES) instruments, next generation sequencing (NGS), and several other instruments.

As well as the analytical sciences, the consortium has a diverse and in-depth practical knowledge in the food sciences, namely fishery, apiculture, animal husbandry, plant growth, vinology, environmental control, natural products, food chemistry and technology, dairy product biochemistry, microbiology, and human exercise biochemistry. Researchers have gained experience from the analysis of thousands of samples of various origin by either untargeted or targeted metabolomics using HRMS; this experience includes reporting solid quantitative data in compliance with validation guidelines. FoodOmicsGR teams collaborate with the industry and the private sector (food
producers, contract research organization (CRO) laboratories, life science companies, and analytical instrument vendors). In addition to that, the groups liaise with international research organizations, regulatory bodies, and consortiums (Eurachem, NIST, mQACC, Norman network, JRC-IRMM, and other key players).

FoodOmicsGR_RI offers:
• Consultancy for the design of experiment (DoE) for the foodomics and nutritional studies
• Genomic, proteomic, and metabolomic analysis of foods
• Quantitative analysis of key metabolites (nutrients, contaminants) in foods
• Big data processing and advanced statistical analysis.

FoodOmicsGR_RI aims to support R&D studies on food metabolomics, proteomics, and genomics applications, offering a “one-stop-shop” model to stakeholders and users.
The Column  www.chromatographyonline.com

also generates compositional databases of the molecular composition of Greek agricultural products. The tools used for this purpose are data- and text-mining of the scientific literature (developing bespoke Python algorithms). The data are soon to be made publicly available on the project webpage.

2. Food Analysis, Traceability, and Control of Geographical Origin: FoodOomicsGR RI research teams have developed and practiced MS and NMR methods for a variety of foods. Untargeted LC–HRMS methods combined with chemometric approaches complement traditional targeted LC–MS analysis (5,6) to study food authenticity.

The consortium has developed MS‑based workflows for food traceability and geographical origin, such as for olive oil, wine, dairy products, and honey. For example, classification of apple varieties was achieved based on volatiles analysis by headspace– solid-phase microextraction (HS–SPME)–GC–MS (7), while the geographical origin of grapes was assessed using a hydrophilic interaction liquid chromatography (HILIC)–MS/MS method (4).

In another study conducted in the laboratory, 63 Cretan virgin olive oils were discriminated according to their geographical origin based on HS–SPME–GC–MS volatile organic compound (VOC) analysis. A representative gas chromatogram of VOCs from agri-food waste, and 6) studies on food safety assessment.

Key R&D axes of the consortium include:

1. Greek Food Composition Databases: One striking example of the portfolio is building in-house high-quality sample libraries establishing and enhancing the nutritional value of Greek foods. The group has published a number of topical reviews on food metabolomics such as olive oil (3), grapes, marc spirits, and wine (4). The group

Figure 4: Principal component analysis (PCA) score plot showing the unsupervised discrimination of Greek red and white wine samples and QC distribution.
from the Koroneiki cultivar is shown in Figure 2. The impact of geographical origin on volatile profile is illustrated in a orthogonal projections to latent structures discriminant analysis (OPLS-DA) model (Figure 3) where samples of different origin are visually separated.

Another interesting example is an untargeted HS–SPME–GC–MS method that was applied for the classification of Greek wines based on their volatile composition. DoE assisted in the optimization of the analysis, while the sample collection consisted of 74 samples of nine different Greek grape varieties and six different regions. Preliminary results indicated that the classification of samples according to grape colour and variety is achievable (Figure 4).

3. Quality Control (QC) and Targeted MS Methods: FoodOmicsGR_RI laboratones have taken key steps and have extensive practical experience in the development of QC protocols for LC–MS-based metabolomics (8,9) for the validation of research findings. The group has studied the effect that sample preparation methods have on the obtained “metabolome” of different samples (10).

After two decades of metabolomics, there is a growing shift towards targeted quantitative methods, as recently reviewed by the group (11). The consortium has developed and validated targeted methods using GC–MS/MS or LC–MS/MS, providing robust and reproducible results for a set of metabolites; these include amino acids (12), organic acids, bile acids, or a wider group of up to 120 metabolites from different small molecule classes (13). Representative examples include studies of royal jelly (13,14), wine (15), and carobs, in addition to biological samples from nutritional studies.

4. Studies on Nutrition and the Nutritional Value of Foods: As stated previously, FoodOmicsGR_RI develops new tools to highlight the nutritional value of Greek foods; this can be attained by performing intervention studies where Greek traditional foods are used in the prevention of chronic diseases and to promote health claims. More specifically, the group monitors the effect of food ingredients at the genomic/transcriptomic/proteomic/metabolic/elemental level, followed by validation of initial findings. Ultimately the group develops proof-of-concept studies on food bioavailability on human organs and tissues. For example, the effect of a carob diet in the metabolome of biological samples of animal models has been studied (16). The facility has expertise in profiling biological fluids, such as urine, plasma, faeces, saliva, and tissues (liver, kidney, brain). Characteristic studies include the effect of diet on human and animal models (16,17,18,19).

Other examples of nutritional studies include collaborations with partners from the private sector to improve health claims of novel superfoods from the Greek flora and marine organisms (that is, use of probiotics to maintain the balance of gut microbiota). Phenolic-rich water extracts from olives and their effect on animals and human health has also been studied. The group has investigated the role of novel animal feed enriched in chelated metals (Cu, Mn, Zn) and identified disease biomarkers in biofluids from sows after livestock intake (Project FITSOW) (20). A key goal is to offer the knowledge that could provide the base for the development of personalized diet based on observations of omics data integration. Such diets could improve health, well-being, and prevent the onset of disease.

5. Assessment of Food Safety

Food safety from chemical contaminants in foodstuffs as a result of environmental contamination, packaging, and storage processes is a major concern in the food market. To this end, the group has studied the migration of an endocrine disruptor (bisphenol A, [BPA]), from baby bottles into the aqueous content of foods or canned food (21). Also, non-intentionally added substances (NIAS) incorporated into the food chain have been studied by various analytical methods, for example, monitoring phthalates (22), oligomers, and other NIAS (23).

Conclusions

FoodOmicsGR_RI aims to characterize unique Greek products and highlight their value and quality using cutting-edge omics technology. The facility promotes and reinforces the collaboration between consortium partners, but, more importantly, it aims to act as a liaison focal point and link the research with the private sector and the producer. Overall, the group’s activity and R&D efforts help to increase the visibility and the market value for the investigated products, support brand naming, and generate higher revenue for the producers and the national economy.

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References

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Messenger RNA (mRNA) vaccines may continue to prove useful in combating new viral variants and more effectively controlling existing strains. However, the analytical approaches for quality control testing must keep pace to ensure the identity, safety, and efficacy of these new prophylactic and therapeutic modalities. This article describes a new approach for measuring critical quality attributes including the confirmation of 5’ cap structure and 5’ capping efficiency, as described herein.

The promise of messenger RNA (mRNA)-based vaccines and therapeutics is being borne out today with the rapid development and emergency authorization of two mRNA vaccine therapeutics designed to fight the ongoing COVID-19 pandemic. Tozinameran (BNT162b2) from Pfizer-BioNTech and mRNA-1273 from Moderna have demonstrated high efficacy against COVID-19 (1), and alternative vaccines are at various stages of development. The success of these two mRNA vaccines has led to a surge of interest, investments, and research into additional mRNA and nucleic acid-based therapeutics. In many cases, the targets for these therapeutics are diseases that conventional drugs cannot treat. One such mRNA candidate currently
in clinical trials is MRT5005 from Translate Bio, which is looking to treat cystic fibrosis (2).

As with all new modalities and as research into mRNA vaccines continues, improvements in areas such as drug delivery and design must occur during the creation of these vaccines (3). Unlike prior vaccines, which include a weakened or inactivated form of the pathogen to illicit immune response, mRNA vaccines provide a set of instructions that receiving cells then translate into a protein. In the case of the COVID-19 vaccines, the protein being produced is the "spike protein" that juts out from the surface of the corona virus. These spike proteins are subsequently recognized by our immune system as being foreign, triggering an immune response and the production of antibodies to neutralize the threat, the result being protection against future infection.

Throughout the process of creating an mRNA vaccine, regulatory requirements for the characterization of mRNA must be met. Though specific requirements from both the International Conference on Harmonization (ICH) and the U.S. Food and Drug Administration (FDA) have not yet been implemented, the FDA and the World Health Organization (WHO) have published guidelines for COVID-19-related vaccines and RNA-based diseases, respectively (4). The WHO draft discusses the evaluation of vaccines and calls out specific critical quality attributes (CQAs), particularly in identifying and quantifying proper cap structure and the poly(A) tail (5,6). Eukaryotic mRNA all contain a cap structure consisting of a 7-methyl guanosine connected to the first nucleotide of mRNA through a 5' to 5' triphosphate bridge. This capping is essential to many functions—for example, for recognizing the mRNA, promoting translation, adding resistance to exonuclease degradation, and more (7).

Depending on the method used to cap synthetic mRNA, different cap structure variants and precursors may be present. Methods to cap mRNA include those that rely on co-transcriptional, post-transcriptional, or enzymatic pathways. As a ubiquitous and highly efficient method, enzymatic preparation of capped mRNA is performed through in vitro transcription using a DNA template, which then produces mRNA with an uncapped triphosphate at its 5' end. Typically, the proper cap structure—Cap-1 for higher eukaryotic species such as humans—can be enzymatically produced from the triphosphate species. Figure 1 details the process of producing Cap-1 from the triphosphate-modified mRNA.

Though enzymatic capping typically produces high yields of Cap-1-modified mRNA, it can be seen from Figure 1 that there are four precursor cap structures throughout the process to form Cap-1. Based on regulatory guidelines, the capping efficiency of the Cap-1 structure must be quantified and any impurities, such as the precursor cap structures, must be identified. Established techniques to analyze these CQAs, in particular the capping efficiency, include variations of enzymatic digestion coupled to gel electrophoresis or chromatography (8). In these methods, the digestion typically produces short strands of uncapped RNA or even mononucleotides to allow an estimation of the percentage of capped sample. However, these methods require the mRNA to be...
labelled, which adds another variable and lengthens the process.

To address this gap in analytics for unmodified mRNA, recent literature has demonstrated the benefits of liquid chromatography–mass spectrometry (LC–MS) analysis to this application, where the identification of non-Cap-1 species can be differentiated through differences in mass and retention time and quantified through MS after digestion of mRNA. Beverly and co-workers from the Novartis Institutes of Biomedical Research employed a biotinylated probe complementary to the 5' end of mRNA RNase H to catalyze the cleavage of pre-determined fragments from the 5' end containing the cap structure. These fragments were enriched and analyzed via LC–MS. In addition, a more current publication in 2021 by Muthmann and co-authors performed digestion of the entire transcript as initiated by nuclease P1, snake venom phosphodiesterase, and dephosphorylation (9). With their method, they generated single nucleosides with or without the cap and quantified these products with triple quadrupole mass spectrometry.

Insights from these works and others show the benefits of implementing an LC–MS method versus an LC optical-only method when measuring CQAs of mRNA, including quantification of the 5' cap fragments. For instance, quantification via UV relies heavily on the separation of the capped species and precursors, whereas LC–MS methods can unequivocally identify and quantify each component, even if not well separated, providing mass certainty as well. To this end, ready deployable, easy-to-use, and compliance-ready LC–MS workflows may prove to be beneficial as the mRNA therapeutic pipeline surges.

Moreover, recently introduced LC technologies have been shown to improve...
the quantification of low-level impurities, for example the 5′ cap precursors leading up to Cap-1. This hybrid surface technology (HST) for column and LC systems hardware addresses and prevents nonspecific adsorption of analytes, particularly acidic compounds, to the electron-deficient metal surfaces of the LC fluid path (10). A highly cross-linked, ethylene-bridged siloxane polymer acts as a barrier that prevents analyte interactions with the metal surface. In particular, the surface technology has been shown to greatly improve the recovery of oligonucleotides, especially at lower level concentrations (10).

**Example of 5′ Cap Structure Analysis with LC–MS**

To demonstrate the benefits of LC–MS in combination with the new LC technologies available, we evaluated an unused hybrid surface column against an unused conventional stainless steel column for the analysis of synthetic RNA oligonucleotides using an ultrahigh-pressure LC (UHPLC) system combined with a simple-to-use time-of-flight mass spectrometer (TOF-MS). These synthetic oligonucleotides consist of 25 nucleotides of the same sequence but are modified with different groups on the 5′ end (pppR-, ppR-, GpppR- or Cap-0, and m7GpppR- or Cap-1). Figure 2 shows the separation and analyte peak of the Cap-1 product using a mobile phase consisting of N, N-dimethylpropylamine (DIPEA) and 1,1,1,3,3,3-hexafluoroisopropanol (HFIP), which has been previously utilized for bioanalytical quantification of oligonucleotides due to its high resolution and MS signal intensities (11,12).

While only the first injection using the hybrid surface column is shown in Figure 2, the peak areas across five injections using this column were reproducible from injection to injection. In contrast, no peak is seen upon first injection using the stainless steel column. As the active sites on the stainless steel surface are slowly passivated by the sample from injection to injection, the Cap-1 peak is finally seen at injection 3. After injection 5, the peak area is still only 37% that of the hybrid surface column. The data suggest that employing inert LC components, such as hybrid surfaces, can improve the sensitivity and reproducibility of 5′ capping identification and quantification assays, and likely many other enzymatic assays where various substrates, precursors, and intermediates need to be tracked and understood.

Notably, from Figure 2, it can be seen that a sub-5-min method was developed to evaluate the capping efficiency of the Cap-1 product. For oligonucleotide separations, historical methods have used longer gradients times. The method developed for the rapid elution of the 5′ capped species shown in Figure 2 employs accurate mass detection to identify the compounds and quantify them through various means, including the use of extracted ion chromatograms. With regards to real world samples, as opposed to the synthetically produced samples shown here, the biotinylated RNAse H probe would also need to be separated from the capped species for suitable quantitation. While not shown...
here, this rapid method can also easily separate a representative RNAse H probe from Cap-1.

To demonstrate the capability of this fast LC–MS method for measuring the capping efficiency of mRNA, we spiked samples of a Cap-1 solution with its four synthetic mRNA precursors (pppR-, ppR-, GpppR-, and m7GpppR-, also referred to as Cap-0) at dilution ratios of 1:10, 1:100, and 1:1000. The Cap-1 concentration was kept at 12.5 pmol/µL. The mass spectra for each dilution is shown in Figure 3. Here, the most abundant charge state for each species including Cap-1 was the [M-10H]^{10−} charge state, which was confirmed by running each individual synthetic RNA fragment and is labelled in Figure 3.

By taking the extracted ion chromatograms (EICs) of the entire isotope mass of each of the [M-10H]^{10−} charge states for the five cap fragments (at 10 ppm), representative calibration curves for the four capped species used to generate Cap-1 can be produced. Even at mass loads of 12.5 fmol (as shown in the 1:1000 EIC for Cap-0 in Figure 4(a)), an EIC can be generated for integration. Figure 4(b) displays the calibration curve data for Cap-0 and its linear regression, with an R² value of 1.000. Though not shown here, the linear regression for pppR-, ppR-, and GpppR- also gave similar R² values, which showcases the potential of MS quantitation for the evaluation of mRNA cap structures.

**Conclusion**
The rapid evaluation of the capping efficiency of mRNA described herein demonstrates the potential of LC–MS for mRNA analysis. Combined with an inert hybrid surface technology applied to the column, LC–MS was capable of assessing the 5’ capping of synthetic mRNA through the relative quantitation of a representative set of data. Coupled to an easy to use, compliance-ready LC–MS workflow, the 5-min method provides mRNA developers and manufacturers with a practical solution for the high-throughput analysis of 5’ cap structure. As the market for mRNA vaccines and gene therapy continues to expand, so will use of enzymatic assays to confirm the relative quantities of representative set of data. Coupled to an easy to use, compliance-ready LC–MS workflow, the 5-min method provides mRNA developers and manufacturers with a practical solution for the high-throughput analysis of 5’ cap structure. As the market for mRNA vaccines and gene therapy continues to expand, so will use of enzymatic assays to confirm critical quality attributes. The development and validation of these assays would benefit from compliance-ready LC–MS workflows that can be deployed across development, production, and quality control (QC) labs.

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**References**

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Three-Dimensional Printing in Ion Mobility and Mass Spectrometry

Interest in three-dimensional (3D) printing technology is expanding rapidly. What impact can it have in mass spectrometry (MS)? Robert Winkler, of the Center for Research and Advanced Studies Irapuato, in Guanajuato, Mexico, is exploring this question. We recently spoke with Prof. Winkler about this work.

—Interview by William Wetzel

Q. What makes three-dimensional (3D)-printing technology valuable in ion mobility spectrometry (IMS) and mass spectrometry (MS)?
A: Before we started using 3D-printing, we spent quite a lot of time building our prototypes. On a daily basis, new exciting ideas for improving analytical instruments are published, and we would like to test some of them. For example, ambient ionization, mass spectrometry imaging, ion manipulation, and portability are very active research areas in ion mobility spectrometry and mass spectrometry. But often, the reported devices are difficult to reproduce and are commercially not available. Using 3D-printing, we can quickly build, modify, test, and share prototypes. This approach also reduces our dependence on large industrial providers, speeds up innovation, and lowers costs.

Q. In a recent article (1), you highlight many recent developments in 3D-printing, including fused deposition modelling (FDM). How can FDM help advance ion mobility spectrometry?
A: FDM is a widespread technology, and simple FDM printers are on sale for a few
The development of bioassays can be tedious because multiple conditions must be tested. Producing cartridges or microreactors for bioassays. What effect has this development had?

Q. Three-dimensional printing has been shown to allow for the fast and cost-effective production of microreactors for bioassays. What effect has this development had?

A: The development of bioassays can be tedious because multiple conditions must be tested. Producing cartridges or microreactor systems with conventional methods is only feasible for extensive studies or routine use. With 3D-printing, a small series of microreactors can be manufactured and enable reproducible experiments. Later, the design of the 3D-printed gadgets can be adopted for mass production.

Q. In your article, you discuss how 3D-printing enables the “peer production” of scientific devices. Can you expand on what “peer production” means and how that connects to the results 3D-printing has achieved so far in ion mobility and mass spectrometry?

A: “Peer production” is the community-driven development and manufacture of goods. Its participants have diverse motivations—not only monetary—for sharing their knowledge. The concept of peer production applies mainly to non-rival goods such as information (3). Wikipedia and Linux are examples of successful peer production, with thousands of volunteer contributors.

We develop analytical devices with public funding and are employed in public institutions. Thus, we should make our results accessible to the people and companies that support our work. If another research group or company adopts our designs, my salary is not affected (“non-rival”), and maybe the users cite us, which helps us justify new project funding. If the situation is the other way around, we also profit from using published files of 3D-printable devices and related literature.

Several groups share their 3D-printing files for IMS and MS parts, either as supplemental material to their papers or on personal request. For example, the files for building or modifying our 3D low-temperature plasma (LTP) probe are freely available online (4). Anyway, providing the necessary files for reproducing 3D-printed prototypes is good practice for publications in analytical chemistry and should be further encouraged by the journals.

Q. What are the major challenges that remain with developing 3D-printing technology for use in mass spectrometry? Have some of these challenges been resolved?

A: Additive manufacturing has various limitations. Some shapes and geometries, for example, overhangs, are difficult to print. Also, the surface quality and spatial resolution of parts may be insufficient for specific applications. Also, not all materials are available for 3D-printing. However, there’s already a wide variety of polymer filaments with diverse properties, such as flexible or conductive.

Three-dimensional printing is a powerful production method, but we have to design printable parts. In some cases, a professional service can print the final component. But often, the combination of 3D-printing with conventional manufacturing methods, and using off-the-shelf parts where possible, leads more quickly to the desired product.

There are also innovative solutions to overcome particular problems, such as the metal coating of 3D-printed parts for making them conductive (5).
high-throughput analyses (6). Now, we are working on a modular miniature mass spectrometer for the real-time analysis of volatile organic compounds (VOCs) and a platform for metabolic phenotyping. Three-dimensional printing plays a central role in the development of our prototypes. Besides, we are creating software to process such multidimensional data.

The possibility of drawing objects in free software and producing them with good quality at low cost motivates students and scientists to bring their ideas to practice. In this way, analytical chemists are not only consumers anymore but are also empowered to create their own devices and share them with the community.

References

Robert Winkler is the PI of the Laboratory for Biochemical and Instrumental Analysis in the Department of Biochemistry and Biotechnology at the Center for Research and Advanced Studies (CINVESTAV) Irapuato, in Guanajuato, Mexico.
The LCGC Blog: The Analytical Arsenal for Point Source Attribution

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How can we monitor the source and environmental impact of neglected oil and gas acquisition sites, and assess their impact over time?

The past year has been a remarkable ride for the oil and gas industry, and for the entire energy sector in general. Extreme volatility in commodity pricing, an oil contango, and an apparent paradigm shift away from fossil fuel-powered vehicles—thanks to Tesla—has all been “par for the course” in the past 12 months. Fortunately, the petroleum industry appears to be making significant strides to improve the environmental stewardship and operational efficiency of energy production by implementing new technologies and capitalizing on previously wasted resources like produced water and flare gas. Nonetheless, many questions still remain about the management of ageing or even abandoned infrastructure because abandoned production wells provide a potential for undocumented contamination events. More specifically, how can we monitor the environmental impacts of these neglected sites, and will these impacted sites grow in magnitude over time? To answer these questions, we must first understand what is required to perform such analyses.

Having contributed to numerous high-profile litigations involving alleged oil and gas-related contamination, we never cease to be amazed at how intricate and idiosyncratic these cases can be. A thorough account of historical events and the chronology of nearby anthropogenic...
activities is certainly helpful, but this information does not tell us about the extent of the problem from an environmental or ecological perspective; and it generally doesn’t provide enough resolution to successfully identify the point of source. For this latter function, we turn to analytical chemistry to help guide us towards a successful outcome, a process known as point source attribution.

In many cases, a relatively straightforward analysis of the environment can provide sufficient insight into the extent of environmental damage from a specific source (1). In the case of alleged groundwater contamination, using ion chromatography and inductively coupled plasma–mass spectrometry (ICP-MS), we can characterize various brine elements (chloride and bromide) and pertinent metal ions species, respectively, to better understand the extent of environmental damage; however, this approach generally doesn’t provide enough resolution to identify the point source. For this latter function, we turn to analytical chemistry to help guide us towards a successful outcome, a process known as point source attribution.

In numerous cases, we have seen that the isotopic signature of the rogue natural gas in a water source is a match with the equivalent signature collected from a nearby production well. Case closed, right? Not exactly. In some instances, there may also be a naturally occurring source of shallow gas that is not economically viable to extract and that has a comparable isotopic signature to the two other thermogenic gases. In situations like these, higher-resolution analytical tools are required to parse out the differences. For example, the relatively recent emergence of noble gas and noble gas isotopic analyses has allowed investigators the opportunity to better perform point source attribution, and simultaneously characterize the migration pathway from the contamination source to the contamination site (2,3). Because noble gases are chemically inert and are not subject to oxidation and microbial degradation, they are ideal tracer elements that can be used to better understand subsurface fluid and gas migration. Using the aforementioned case with three matching methane isotope signatures, the characterization of noble gas analytes allowed us to differentiate water wells that had been inadvertently drilled directly into the intermediate gas layer from those that has been impacted by the activities of a nearby production well (4).

Another tool that is adding a lot of value to environmental investigations, particularly groundwater and surface water analyses, is matrix-assisted laser desorption–ionization time-of-flight mass spectrometry (MALDI-TOF-MS). This particular tool has made a tremendous impact in the clinical setting as a way to screen through a large library of bacterial species that can have deleterious effects to susceptible or immune-compromised patients. MALDI-TOF-MS is now making a big splash in the environmental realm with the ability to screen samples for the presence of thousands of different microbes (5). Our research across Texas using MALDI-TOF-MS has demonstrated the value of comprehensive microbial analyses as a way to characterize bacterial ecology, which can be representative of the current contamination state (6,7). And although the aforementioned noble gas analyses are arguably the most powerful weapon in the environmental arsenal, MALDI-TOF-MS microbial analysis is much more rapid (<48 h) and can detect various indicator species that can suggest the presence or absence of atypical environmental conditions. For example, the detection of extremophilic or halophilic species that are not native to typical, healthy groundwater conditions can be indicative of a much larger problem that may warrant further investigation.

Unconventional oil and gas extraction is a multi-faceted landscape. From assessment of air, soil, and water quality to oilfield wastewater treatment and re-use, there are many places where both routine and advanced chemical analysis can provide insight. Even as some favour the rapid transition to renewable energy resources, the extraction and use of fossil fuels will continue as a pertinent piece of the energy supply puzzle for the foreseeable future. We will continue to be faced with situations where environmental
forensics and point source attribution are needed to understand the cause of various contamination events. Even if fossil fuel extraction declines significantly over the next 20 years, we will still be left with the need to ensure abandoned well sites are properly closed and pose no threat to the environment. We have the technology and the know-how, but of course, there are still real limits in the research that can be performed with limited to no investment in such studies from traditional funding agencies.

References


This blog is a collaboration between LCGC and the American Chemical Society Analytical Division Subdivision on chromatography and separations chemistry.

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Het Pand Conference Center, Ghent, Belgium
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Website: https://htc-17.com/

5–9 March 2022
Pittcon 2022
Georgia World Congress Center, Atlanta, Georgia, USA
E-mail: info@pittcon.org
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11–14 October 2022
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